UNIVERSITY OF SOUTHAMPTON

THE ASSESSMENT OF SPERM VELOCITY AND PROLACTIN IN THE INFERTILE MALE

by

MICHAEL PETER MILLIGAN
M.B., B.S., M.R.C.O.G.

Thesis submitted for the Degree of Doctor of Medicine

December, 1980

Resubmitted

September, 1981.

TABLE OF CONTENTS

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE HUMAN REPRODUCTION AND OBSTETRICS

Doctor of Medicine

THE ASSESSMENT OF SPERM VELOCITY AND PROLACTIN IN THE INFERTILE MALE

by Michael Peter Milligan

The first part of the study comprises the development of a method of measuring sperm velocity (μm/sec) by time-lapse photography (TLP). Recordings were performed in 50 infertile men and compared to 20 fertile men. TLP was found to be an accurate method of measuring sperm velocity with a low coefficient of variation. Its accuracy is limited at low sperm density levels (less than 5 million/ml). Sperm velocity is a separate parameter from percentage motility and sperm density. It is not dependent on serum hormonal levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), 17β oestradiol and dihydrotestosterone (DHT).

A statistically significant difference in sperm velocity was found between the two groups studied and no fertile male had a sperm velocity of less than 30 μm/sec. The importance of sperm velocity as a new parameter in the investigation of male infertility is discussed. A statistically significant difference in the levels of serum 17β oestradiol was also noted between fertile and infertile men.

The second part studies the role of serum prolactin in spermatogenesis using the same infertile (50) and fertile (20) patients. There was no statistical difference in either serum or seminal prolactin between the two groups. In the infertile patients FSH levels were found to correlate with prolactin at both serum and seminal levels. Serum LH was also correlated with seminal prolactin. No such correlations were found in the fertile group.

The 50 infertile patients were randomly separated into two groups and received Bromocriptine 2.5 mg b.d. or placebo on a double-blind parallel group basis. The patients were re-assessed after three and six months' treatment.

Bromocriptine is an effective prolactin-lowering agent at both serum and seminal level. Bromocriptine has a deleterious effect on sperm production and no apparent effect on androgen metabolism.

Dihydrotestosterone (DHT) progressively rose in both placebo and Bromocriptine treated groups and it is suggested that this might explain the apparent beneficial effect on spermatogenesis by placebo noted in other series.

ACKNOWLEDGEMENTS

I should like to acknowledge the help of the many people who, in numerous ways, have assisted with the work and with the preparation of this thesis.

I should especially like to thank Professor K. John Dennis for his encouragement, guidance and patience. Without the support and facilities of the Department of Human Reproduction and Obstetrics this thesis would not have been possible.

In particular I should like to thank Dr. Graham Kennedy of Sandoz and Sandoz Limited who provided the financial support for this project over the past three years.

I am also indebted for the help of Dr. P. Wood and Dr. A. Evans of the Department of Chemical Pathology, Southampton General Hospital, who kindly carried out the assays; Mr. M. Harman, Department of Community Medicine for assistance in computer analysis and statistics; Mr. S.J. Harris for assistance in semen analyses; Mr. F. Anthony for assistance with the oestradiol assays; the Consultant Obstetric staff of Southampton General Hospital for access to their patients; Dr. A.T. Letchworth for his encouragement; Professor M. Elstein; Professor J. Birkbeck for proof reading the manuscript; Mrs. K. Morris for preparing this manuscript and making an impossible task possible.

DECLARATION

I hereby declare that the whole of the work submitted in this thesis is the result of my own observations, except where reference is made to published literature and where assistance is acknowledged.

No part of this thesis has previously been submitted for consideration for a degree.

[redacted]

M. P. Milligan.

FORM OF REFERENCE ANNOTATION

A modified Harvard system of reference annotation has been used throughout this thesis. On occasion when one author has presented more than one paper in that year the references have been marked alphabetically. Where more than two authors are involved, only the first is mentioned in the text.

DEFINITIONS

Klinefelter's Syndrome

A condition characterized by the presence of small testes, with fibrosis and hyalinization of seminiferous tubules, without involvement of Leydig cells, and by increase in urinary gonadotrophins; associated with an abnormality of the sex chromosomes.

"Sertoli cell only" Syndrome

The absence from the seminiferous tubules of the testis of germinal epithelium, Sertoli cells alone being present.

Sperm Count/Density

The number of spermatozoa expressed in millions per millilitre of seminal fluid.

Percentage Abnormal Forms

The number of abnormal spermatozoa in the ejaculate expressed as a percentage.

Percentage Live/Dead

The number of dead spermatozoa in the ejaculate expressed as a percentage.

Percentage Motility

The number of motile spermatozoa in the ejaculate expressed as a percentage.

Percentage Live Motility

The number of motile spermatozoa in the ejaculates expressed as a percentage of the live cells present.

Normospermia

Sperm density in excess of forty million per millilitre of seminal fluid.

Oligospermia

Sperm density below forty million per millilitre of seminal fluid.

Asthenospermia

A percentage motility below fifty per cent.

Azoospermia

Total absence of spermatozoa in the seminal fluid.

Sperm Velocity:

The average speed of the motile spermatozoa expressed in microns per second.

ABBREVIATIONS

TLP	Time Lapse Photography
FSH	Follicle Stimulating Hormone
LH	Luteinizing Hormone
DHT	Dihydrotestosterone
LRH	LH/FSH Releasing Hormone
T_3	Triodothyroxine
T_4	Thyroxine
ATP	Adenosine-5-triphosphate
AMP	Adenosine-3'-5'-cyclic monophosphate
ASA	American Standards Association (film speed)
IU/l	International Units per Litre
mIU/l	Milliunits (international) per Litre
nmol/l	Nanomoles per Lites (10^{-9}mole per litre)
pmol/l	Picomoles per Litre (10^{-12}mole per litre)
ng/100 ml	Nanograms per Hundred Millilitres (10^{-9} grams per 100)
µmol/l	Micromoles per Litre (10^{-6} per 1)
ml	Millilitre
cc	Cubic Centimetre
µg/ml	Micrograms per Millilitre (10^{-6} grams per ml)
µm/sec	Micrometres per Second (10^{-6} metre per sec)
g	Grams
^{o}C	Degrees Centigrade
10^6	Millions
10^6/ml	Millions per Millilitre
cms	Centimetres
%	Percentage

revs	Revolutions
$<$	Less than
$>$	Greater than
n	Number (of observations)
SD	Standard Deviation
t -	Student's "t" test
p	Probability
f value	Variance ratio
pH	Hydrogen ion (H^+) concentration
vs.	Versus

STATISTICAL ANALYSIS

A modified Students 't' test was performed. This incorporated an F test in view of the possible difference in the variance of the two populations being studied (fertile and infertile). Depending on the F value either a separate variance estimate or a pooled variance estimate was used to provide the degree of statistical difference. A significant level of 5% was used throughout the thesis (p 0.05) and the actual p value as calculated is recorded in the text.

In correlation to other parameters, both parametric and non-parametric scattergrams were performed. Since there was no difference in either Part I or Part II in the statistical significance calculated in this manner, only the parametric values were included in the text. This fact is particularly important in the statistical analysis carried out in Part II, where obvious skewed distribution appeared in some parameters.

In Part II, when analysing the effect of prolactin lowering agent (Bromocriptine) paired 't' tests were carried out and the p values as calculated are recorded in the text. Pairing was used in this case since the effect of the agent being studied was less than the sample-to-sample variation. Again a significant level of 5% was chosen.

PART I

THE ASSESSMENT OF SPERM VELOCITY IN THE INFERTILE MALE

CHAPTER I

INTRODUCTION AND AIMS OF STUDY

ASSESSMENT OF THE INFERTILE MALE

- A Review of the Literature

His seed shall be mighty
upon earth: the generation
of the upright shall be
blessed.

Psalm 112: 2.

INTRODUCTION

"The contribution of male factors which cause an infertile marriage has been recognised more readily with the increasing awareness of clinicians of this problem".

This statement from de Kretser (1974) summarises the position that pertains to the last five years in the area of male reproduction. He went on to estimate that approximately one in ten marriages was infertile and that his figures identify that male factors contribute significantly in thirty-five to forty per cent of such marriages. While advances have been made in the understanding of many aspects of normal male reproductive function, the pathogenesis of the majority of disorders resulting in infertility remains obscure. Furthermore, the advance in knowledge concerning hormonal agents controlling spermatogenesis, the kinetics of that process and the cytogenetic factors influencing sperm production have just started to produce a revised concept of the control of male fertility. The processes which go together to make a normal fertile male are multifactorial and only those parameters which are now accepted in clinical work will be discussed in this Thesis. However, these accepted parameters unfortunately only have a poor correlation to the potential fertility of the male partner, thus making identification of a possible defect extremely difficult and in consequence making evaluation of new drug regimes designed to correct such defects, extremely unrewarding.

The basis of this Thesis therefore, was to try and establish a new parameter in the assessment of male infertility and once established, to test it against the possible role of prolactin in spermatogenesis.

AIMS OF STUDY

The aims of this study are to ascertain:

Whether sperm velocity might be a better parameter in the clinical assessment of fertility.

Whether sperm velocity correlates with any other presently accepted parameter in male infertility.

Whether human prolactin plays a significant role in spermatogenesis and whether the use of Bromocriptine as a prolactin-lowering agent can affect fertility.

PRESENT METHODS FOR ASSESSING POTENTIAL FERTILITY IN THE MALE

Review of Literature.

At the present time the potentially infertile male is assessed at clinical examination to exclude gross defects and anatomical anomalies. In addition, the following tests are performed:

(1) Seminal analysis in terms of sperm density, volume, morphology and percentage motility.

(2) Serum hormonal estimation such as FSH, LH and Testosterone.

Seminal Analysis:

(a) Sperm Density:

Originally sperm density was used as the one parameter by which to adjudge a male's fertility index. It is readily obtained, easy to measure and is therefore an economical test. The importance of the sperm density was established in a series of papers written between 1951 and 1956 by Macleod & Gold. In their 1951 paper they established the differences in density counts between fertile and infertile marriages and also established the normal ejaculatory volume. It was a comprehensive work looking at the relative frequency distributions and they came to the following very important conclusions:

1) The median ejaculatory volume for a fertile group was 3.20 cc.s and for an infertile group, 3.50 cc.s. This difference was said to be significant, but no p value was given.

2) The median spermatozoan counts per cc for a fertile group was 90 million and for an infertile group, 74 million.

The real difference between the two groups in this series, lies in the relative frequency distributions and only at the lower levels - i.e. 20 million per cc or below, was there a significant difference between the fertile and infertile groups. This was in itself an advance, since up to this time, much higher counts were being accepted as the lower limit of normal in the assessment of the infertile male.

Naghma-e-Rehan (1974) reported on 1300 fertile men in a careful statistical analysis. In this series a sperm density was reported for fertile men (pre-vasectomy group) of a geometric mean of 65 million with a distribution from 30 million to 142 million. This figure was significantly lower than that of Macleod ($p < 0.01$). The patients studied in this series - and thus the criteria for selection - came from those men presenting for vasectomy and who had already fathered a child.

Eliasson (1971a) however, chose a much stricter definition of fertility and only used specimens taken from a small group of

29 men within three months of their wives becoming pregnant. This series reported a mean count of 86 million/ml which was in agreement with the original Macleod figures. However, in view of the different criteria for selection of their patients, any comparison of the above data is meaningless.

Van Zyl (1976) suggested an even lower level at which significant differences would appear between fertile and infertile groups and in his series, a difference was only found at the level of 10 million/ml or a total count of 25 million.

In spite of the above information, the World Health Organisation definition of oligospermia is a sperm density of less than 20 million/ml. On the basis of this obviously ill-defined parameter, sperm density has been used as an important marker of male fertility by which the effectiveness of any particular treatment is judged. Because of this many drug therapies have been suggested and although they have been shown to increase sperm density, they have not increased pregnancy rates by a similar margin. A concurrent lack of properly defined control groups, makes the statistical analysis of such data less meaningful.

(b) Sperm Morphology:

Although the definition of a normal spermatozoon may be beyond dispute, morphological examination of the spermatozoa is subjective. This means that the tradition of analysis may

develop in different directions in different laboratories and this was indeed shown by Freund (1966). However, due to an initiative by Eliasson (1971b) to improve the situation, the criteria of abnormal spermatozoa are now more accurately defined. However, in spite of this there are strong grounds to suggest that the laboratory data with regard to sperm morphology cannot be accurately translated from one laboratory to another. When different laboratories have carried out morphological assessment of the same ejaculate, very wide differences have been shown (Fredricsson, 1979). This underlines the problems of trying to compare the results of two different centres. However, two things have been accepted in this field; firstly that the presence of more than 25% abnormal forms is considered to be abnormal and secondly, the appearance of immature forms with a higher number of abnormal forms suggests some severe disorder of spermatogenesis (de Kretser, 1974). A number of factors can affect morphology, such as constitutional illnesses and the time between the last ejaculate, since a longer period of abstinence is known to produce an ageing effect and to increase the percentage of abnormal forms (Macleod, 1951). It has also been suggested that chromosomal abnormalities in the male genotype can affect sperm morphology, although this evidence has not been established. An increased number of abnormal forms has also been suggested as a cause of repeated first-trimester miscarriages. Again, like so many parameters of male infertility, these hypotheses have not been studied with carefully matched parallel group studies and therefore the role of sperm morphology as an assessment of potential fertility is difficult to evaluate.

(c) Sperm Motility:

Motility is thought by many workers to be the most important single factor in determining male fertility. Sperm motility is traditionally expressed as a percentage and is a subjective figure with considerable individual variation. However, the ability to move does indicate a certain maturity, although this motility does not necessarily mean that a sperm is able to fertilise. Mature sperm are held in the epididymis and it is only on the actual ejaculation that the motility is established. Indeed, severe damage to the epididymis - whether it be by trauma or infection - can cause permanent reduction in sperm motility.

The actual process by which mature sperm obtains its motility is a complicated biochemical and biophysical process, much of which is unknown. What is known is that ATP is the main energy source for sperm motility in initiating and propagating the sperm flagellar wave. Endogenous respiration is mainly by the oxidation of intra-cellular lipids. Metabolism of extra-cellular glucose and fructose is coupled with ATP formation. Cyclic acid nucleotides mediate initiation and control of flagellar wave formation. The two major cations - calcium and magnesium - regulate the activity of the enzymes involved in flagellar contraction and relaxation.

The morphological organisation of the sperm flagellum consists of nine coarse outer fibres containing the contractile

proteins flectin and spermosin strategically placed for the propogation of the flagellar wave. The final velocity is known to be dependent on the kinetic energy overcoming the internal resistance to bending and the viscous drag of the fluid. On ejaculation the spermatozoa are mixed with seminal plasma and the inhibition on their motility is removed. The final motility is dependent upon the ratio of the prostatic fluid's stimulating motility while the vesicular fluids have a deleterious effect. The motility of spermatozoa is probably only secondary to other transport mechanisms, such as muscular contractions and ciliary motion in the female reproductive tract, in its role of transportation to the site of fertilisation. However, such motility is of prime importance in the passage to the cervix and the utero-tubal junction and most important in the actual penetration of the cumular cells and zona pellucida of the ova immediately prior to fertilisation.

In spite of the accepted importance of sperm motility by clinicians, very few series have shown a direct relationship between this parameter and potential fertility. Farris in 1949 studied the semen specimens from 239 individuals and correlated number of active sperm in total ejaculates to reproductive performance of donors. Those containing sperm counts greater than 185 million were highly fertile, specimens containing 80 to 185 million active sperms were relatively

fertile, but those containing less than 80 million active sperm were subfertile. In this series the wives of men who possessed greater active sperm counts in the entire ejaculate, conceived more easily than the wives of men with few active sperm in the ejaculate.

Harvey & Johnson (1945) showed that there was an increased number of immotile spermatozoa following long periods of sexual abstinence. Further work by Macleod (1951) showed that this decrease in seminal quality was more apparent in subfertile than fertile patients. These two series show that sperm motility is influenced by the time between ejaculations.

Van Zyl (1972) demonstrated the existence of a direct linear relationship between conception rate and percentage motility. In this series the greater instance of pregnancy occurred when motility exceeded 70% and none occurred when it fell below 10%. Santomauro (1972) studied 358 couples, 79 of which were fertile. Of the infertile couples the incidence of the male as the sole cause of infertility was 36.4%. He showed a significant difference between the two groups in terms of sperm density ($p = < 0.01$). Approximately 38% of the infertile males compared to 13% of the fertile males showed less than 40% motility.

However, in the Naghma-e-Rehan series (1974) which was

only studying apparently fertile men, the percentage of active sperm varied from 5 to 95%. The mean percentage of the active sperm was found to be 65% with a standard deviation of 22%. 14% of his patients had a motility of less than 40%. In this series there was a definite positive correlation ($p < 0.02$) between sperm density and percentage of motile sperm. Page & Houlding (1951) also found a relationship between motility and sperm count in 129 fertile men they selected from their series of 1000.

In spite of what appears to be a fairly well demonstrated connection between sperm motility and potential fertility, the subjective nature of this test with its large individual percentage error cannot be avoided. Motility is essential for fertility, but it must be stressed that it is not necessarily indicative of a fertilising capacity. Normal spermatozoa lose fertilising ability long before they lose motility (Harvey, 1960). She also showed that abnormal spermatozoa can exhibit normal motility and yet be incapable of fertilisation.

In summary it appears that sperm motility is indicative of a certain maturation and it is an essential parameter for fertilisation. However, its method of estimation is imprecise and it is for that reason that a method for estimating velocity of spermatozoa has been developed and described in this Thesis.

Serum Hormonal Estimations:

(a) Follicle Stimulating Hormone:

The recent development of specific and sensitive radioimmunoassays for the measurement of the hormones involved in male reproduction have enabled hormonal profiles of patients presenting with infertility to be thoroughly assessed (de Kretser, 1972; Leonard, 1972; Franchimont, 1972). The results of these studies demonstrated that in the majority of patients with oligospermia or azoospermia who had adequate levels of FSH and LH, a gonadotrophic deficiency did not play a role in the pathogenesis of these disorders. Jackaman et al (1977) demonstrated an increase in the serum level of FSH in all men with depressed spermatogenesis and later attempted to correlate FSH directly with spermatogenesis based on the histological findings at testicular biopsy. They showed a positive correlation between sperm concentration and testicular histology. They also showed that the more severe the structural changes, the more significant were the differences compared with the normal. In particular, tubular hyalinisation and Sertoli Cell Only Syndrome (histology shows complete lack of spermatogenesis) had a strong positive correlation with high FSH levels. They finally concluded in this paper that men with azoospermia and a FSH level 3 - 4 times greater than normal would have severely distorted histological changes and that testicular biopsy would therefore be largely academic. However, in situations with slightly

lower values of FSH, a wide range of impaired spermatogenesis could be present and therefore suggested testicular biopsies would still be useful. De Kretser (1972) suggested that serum FSH levels were significantly related to the numbers of germinal cells, in particular spermatogonia. He also agreed with the previous high elevations of FSH in cases of tubular hyalinisation and Sertoli Cell Only Syndrome. Franchimont (1972) showed FSH to be significantly elevated with spermatogenesis arrested before the stage of spermatid formation. Similarly, Hunter (1974) showed high FSH levels associated with profound histological defects, while Bramble (1975) emphasized that the high base levels of FSH indicated germinal cell aplasia or arrest.

In contrast, low levels of FSH and LH are found in patients with hypogonadotrophic hypogonadism, pituitary tumours or following hypophysectomy (Paulsen, 1965; Gemzell & Kjessler, 1964; Johnsen, 1966; Macleod, 1966). Dynamic tests of the hypothalamic pituitary axis in these such patients with the use of Clomiphene (Santen, 1971) and more recently with LRH (de Kretser, 1972) has established a rare group of men who show isolated FSH deficiency. This failure to react to an LRH challenge test has further been shown by Lunenfeld (1974). De Kretser (1974), suggested that there was a much greater fluctuation in the levels of FSH as well as LH and Testosterone in serum and suggested a 24-hour collection for the evaluation might be more accurate. Spera and colleagues (1978) concluded

that on reviewing the previous literature, although there was agreement that high levels of FSH were associated with a severe disorder of seminiferous epithelium function, there was no agreement in the role played by various elements of the germinal epithelium in the production of an FSH inhibiting factor. They set out to study the relationship between plasma FSH and LH and the degree of seminiferous epithelial impairment in infertile men, grouped according to histological changes. An LRH test was performed in some patients in order to evaluate the difference between FSH and LH response in patients from each group. The results led them to the following conclusions:

They suggested that the plasma levels of FSH are influenced by seminiferous epithelium, but that the mechanism responsible was then unknown. They did suggest that late spermatids are fundamentally involved in the production of an FSH inhibiting factor. In the absence of late spermatids, two phenomena occurred. Firstly, basal FSH levels are increased and secondly, FSH appears to be liberated following LRH stimulation, showing a similar response to that of LH. Moreover, they showed that the number of late spermatids regulate the basal production FSH and thus the efficiency of spermatogenesis. They postulated that spermatocytes and spermatogonia influence the production of an FSH-inhibiting factor which depends upon the ability of the spermatozoon to mature as far as the spermatid. In cases of spermatocytic or spermatogonial arrest, the basal levels of FSH are significantly higher than in cases of oligospermatogenesis and also that the FSH response to the LRH challenge test shows

an increase similar to that of LH. At the same time they confirmed the importance of Sertoli cells in the regulation of FSH production which had already been demonstrated by Heath (1973) and Davies (1976). This FSH inhibiting factor is now referred to as Inhibin and plays an important part in the feedback mechanism in the regulation of FSH release. Fossati (1979) although showing that the mean basal FSH levels were higher in the oligospermic group than in a normospermic group noted no correlation in either group between basal serum FSH and sperm density. This apparent contradiction to the previous literature was put down to different composition of groups of patients being studied and the different hormonal assays used.

In summary it can be said that FSH plays an important role in the stimulation and maturation of the individual spermatozoon. At the spermatid level, FSH inhibiting factor or Inhibin, is released, which has a negative feedback effect on the release of FSH. Serum FSH levels more than 2 - 3 times the normal indicate a severe disorder in spermatogenesis and high levels of FSH are directly related to the number of spermatogonia.

(b) Luteinizing Hormone (LH):

The relationship between luteinizing hormone (LH) and spermatogenesis however, is less well defined. Franchimont (1972) found no correlation between Serum LH and either the sperm count or stage of maturation of spermatogenesis. In contrast Hunter (1974) did report a correlation between LH and sperm count in

285 patients. Jackaman (1977) showed that although serum testosterone is directly related to LH for a feedback mechanism, this relationship in the subfertile male still required further investigation. Low levels of testosterone with elevated LH levels in serum can be found in approximately 30% of males with testicular disorders, according to de Kretser (1974). He went on to show that in these men the response of plasma testosterone to stimulation with human chorionic gonadotrophin is often subnormal, which is a further index of an abnormal interstitial cell function. Jackaman (1977) also showed that the serum FSH level was found to be elevated in patients with focal tubal atrophy and also Sertol Cell Only Syndrome. He stated that the previous studies by Franchimont (1972) and de Kretser (1972) had shown that the serum LH gave no conclusive correlation with testicular histology. In his paper he suggested that their findings showed a synergistic effect of LH and FSH and also a gradual increase in their levels with the severity of the testicular lesion. In patients with Sertoli Cell Only Syndrome who have severe testicular damage, FSH was shown to be considerably raised, while the LH was only moderately raised. However, in focal tubal atrophy, FSH is high while LH only slightly higher than normal.

Spera (1978) could find no significant correlation between LH levels and severe impairment of seminiferous epithelium, while Fossati (1979) showed that the mean basal LH value was elevated in an oligospermic group compared with a normospermic group. This finding was in agreement with the increased urinary LH levels in

oligospermics, but again he stressed that no similar correlations were found between basal serum LH levels and the sperm count in either group.

Therefore, in summary, there seems to be no direct correlation between serum LH levels and the degree of impairment of spermatogenesis. Particularly severe disorders of testicular histology are associated with mildly elevated levels of LH. However, LH is known to be important in the function of the Leydig cells and the subsequent production of testosterone. The subsequent negative feedback mechanism of testosterone on LH secretion - whether direct or via conversion to oestradiol, is not fully evaluated.

(c) Serum Testosterone:

Surprisingly little research work has been carried out to discern the place of serum testosterone and its role in the assessment of the infertile male. Although testosterone is known to be directly related to LH through a feedback mechanism as already described, its actual significance as a clinical parameter in the subfertile male, remains unclear. In 1971 Rosen & Weintraub showed that in patients presenting with Kleinefelter's Syndrome, the serum testosterone levels were reduced but not significantly. De Kretser (1972) when studying a relatively large number of infertile patients grouped according to histological findings and testicular biopsy, showed no significant difference in the serum testosterone between any of the groups. Jackaman (1977) looked at 137 subfertile patients to determine the relationships between, as

already stated, FSH, LH and now testosterone levels. He also showed that there were no significant differences in the serum testosterone levels between any of the histological groups he studied. These included focal tubal atrophy, maturation arrest, Sertoli Cell Only Syndrome and apparently normal histology. He concluded that there was little value in the routine measurement of testosterone in the infertile male and underlined that the role of testosterine in such patients was yet to be determined.

Suominen (1979) looking at prolactin levels in azoospermic men and in relationship to various parameters, showed that high prolactin values were found in patients who had a low serum testosterone. This finding was in accordance with Segal (1976) and Krause (1977). They concluded that the increased prolactin levels were usually associated with low testosterone values, although no possible explanation was given for this apparent relationship. It seems therefore, that the use of serum testosterone as a parameter in the assessment of the infertile male, is of little use, apart from extreme situations such as Klinefelter's Syndrome. There is certainly no other evidence in the literature presently which contradicts this view. Serum testosterone, however, is only one marker of androgenic function. The most active androgen in the seminiferous tubules and epididymis is known to be dihydrotestosterone (DHT) metabolised from testosterone. For this reason DHT and not testosterone was studied in this thesis.

DIHYDROTESTOSTERONE (DHT)

Although dihydrotestosterone has yet to be used as a parameter of male fertility, information does exist with relationship to spermatogenesis. De Aloysio et al (1974) showed that the plasma and semen concentration of testosterone decreases progressively in relationship to the decreasing number of spermatozoa present in semen. On the basis of this paper, he went on to investigate the plasma and seminal concentrations of various androgens in normospermic and oligospermic subjects. His definition of oligospermia was less than 40 million/ml when he looked at a total of 120 subjects. At the plasma less level, testosterone concentration tended to diminish when the number of spermatozoa was reduced, whereas 5α-dihydrotestosterone at Δ_4-androstenedione did not show appreciable changes. At the semen level the concentrations of testosterone and 5α-dihydrotestosterone were reduced and the number of spermatozoa was distinctly low. However, Δ_4-androstenedione did not undergo relevant changes. As far as the plasma-semen ratio was concerned, 5α-dihydrotestosterone was increased as a function of the reduced number of spermatozoa, while the ratio for Δ_4-androstenedione was unchanged. Further to this, 5α-dihydrotestosterone showed similar concentrations in plasma and seminal fluid, unlike the two others, testosterone and androstenedione.

His main conclusion was that the androgens measured did not appear to have a precise correlation to the number of spermatozoa present in the semen. However, he did point out that there was a trend, albeit not significant, in the

relationship between the androgens measured, especially at the seminal level, to the reduced number of spermatozoa, though this was not so for Δ_4-androstenedione concentrations which appear from this paper to be independent of tubule activity and to be related to adrenal function instead. He finally concluded that 5α-dihydrotestosterone would appear to be the most important androgen in the seminal fluid, where its relevant concentration was higher than that of the other two substances.

Most of the previous work in this field had been carried out in rats and in Harris's paper in 1977, she pointed out that although spermatogenesis of the rat appears to depend primarily on high local concentration of testosterone (Steinberger, 1975) the action of testosterone may involve its reduction to dihydrotestosterone. Steinberger (1974) had suggested this possibility due to the mechanism of testosterone action in the accessory reproductive glands and by the observation that testicular androgen receptors and testicular androgen-binding protein had high affinity for both testosterone and dihydrotestosterone. Rivarola (1975) had already shown the presence of 5α-reductase in the seminiferous tubules and Chemes (1976) had shown that the administration of dihydrotestosterone can initiate spermatogenesis treated with oestradiol. In Harris's study in 1977, the degree of maintenance of restoration of spermatogenesis in adult hypophysectomised rats was slightly greater after treatment with testosterone than after treatment with dihydrotestosterone, and that this difference was associated with significantly higher

peripheral androgen levels in the testosterone-treated animals, as compared to the dihydrotestosterone-treated animals. This difference in peripheral androgen levels is most likely, she claimed, due to the fact that in rat, the metabolic clearance rate of dihydrotestosterone is much greater than the metabolic clearance rate of testosterone (Lee, 1975; Van Doorn, 1975). However, she did point out at that time, no information was available on the metabolism of these steroids by subcutaneous tissues. She went on to point out that androstenediol can also restore and maintain spermatogenesis (Chemes, 1976) because of its limited conversion to DHT within the seminiferous tubules. However, she rightly pointed out that any comparison of the biologic properties of testosterone and dihydrotestosterone is further complicated by the striking species differences in the rate of dihydrotestosterone metabolism, (Gay, 1976) and by the differential ability of these steroids to cross the blood-brain barrier (Marynick, 1976).

Her final conclusion was that information to that date did not point to which of the two androgens is of greater importance at cellular level in the rat.

Le Lannou reporting in 1980 looked at testosterone and dihydrotestosterone in human seminal plasma. He again showed that at seminal level, there was no significant difference in testosterone concentration between the fertile and infertile seminal samples. He pointed out the difference between these two androgens and their relationship at plasma and seminal level. He showed that there was a correlation between

testosterone and dihydrotestosterone in circulating plasma, thus confirming the work of Purvis et al (1975). Fiorelli (1976), noted that DHT mostly derives from the peripheral conversion of testosterone. However, Le Lannou suggested that the blood-testis barrier between the two compartments limits the transfer of certain steroids from blood to semen. Testosterone is known to cross the barrier more easily, whereas DHT does so in very small amounts (Setchell & Main, 1975).

At the seminal level, DHT can be regarded as the most important androgen in the male genital tract and as Le Lannou pointed out, high concentrations of DHT have been reported in the epididymis, but its origin is unclear. In the human, DHT concentrations are higher in the epididymis than in the testis (Purvis et al, 1976). Le Lannou finally concluded that testosterone derives essentially from the accessory sex glands, whereas DHT is mainly of testicular or epididymal origin. He went on to suggest that the low DHT concentrations found in seminal plasma of oligospermic and azoospermic patients, is probably due to the defective epididymal conversion of testosterone into DHT.

PLASMA 17β OESTRADIOL

Plasma 17β Oestradiol was included in this thesis in view of the recent possibility that oestradiol may be involved in spermatogenesis. In the rat, information exists suggesting that testosterone is metabolised to both dihydrotestosterone and oestrogen (Purvis, 1977; Dorrington, 1978). Franchimont in 1975, showed that oestradiol and inhibin originate from the Sertoli cells and are said to have a selective effect on secretion of FSH and Labrie (1978) showed that oestrogens have the potential of directly affecting the pituitary responses to LRH, along with androgens and inhibin.

Mulder et al in 1976 showed that oestradiol is produced by the testis and that a high concentration of specific receptors for this steroid is present in the Leydig cells. However, the specific role of this steroid in the control of spermatogenesis is still controversial. Oestradiol administration is known to induce a decrease in plasma testosterone and atrophy of accessory sex glands, together with a reduction of plasma LH levels (De Jong, 1975) in rats. The administration of LH can overcome the inhibitory factor of oestradiol on testosterone, indicating that this inhibition is mediated by the pituitary (Van Beurden, 1977). However, other experiments have shown a decrease in testosterone production after oestradiol administration in isolated Leydig cells in vitro (Sholiton, 1975). As Tresguerres pointed out in 1980, these results suggest the hypothesis of direct action of oestradiol at the testicular level. However, he

did correctly point out at the conclusion of his paper, that the mechanism involved in the suppressive action of oestradiol on the testis was a complex one and there was a need for many more experimental approaches to elucidate the exact mode of action.

REVIEW OF THE LITERATURE IN THE ASSESSMENT OF SPERM VELOCITY

Motility is a characteristic of the sperm cell which is so readily observable that it is understandable that it was a convenient indicator of the physiological state of the cell. The actual method by which the spermatozoon obtains its motility has already been discussed in Chapter I, but there is no firm correlation between motility and the fertilising capacity of the spermatozoon. The determination of changes in the percentage of motility is easy to assess in human semen, but is subject to considerable error. Correct assessment of motility is qualitative rather than quantitative and is expressed as a percentage; when only one observer is utilised in a particular series, one can expect to find a reasonable degree of accuracy. Individual error however, makes comparison of one series to another, difficult. Two specimens with the same percentage of motility in them can, on observation, give a completely different clinical impression; for instance, in a situation where there is a considerable amount of antisperm antibodies causing severe agglutination, the percentage motility is greatly reduced, but those which are free can be highly active, thus suggesting good capacity for fertilisation. Alternatively, with a high percentage motility, there can be little in the way of progression of each individual spermatozoon, suggesting a relatively infertile specimen.

In view of this, many workers looked for other methods of quantivating the parameter of motility. It became apparent that the quality of movement is probably at least as important as the percentage motility as a measure of the potential fertility of semen. It seemed therefore desirable to devise a method for estimating the speed of movement of spermatozoa.

The methodology to make this possible started with the work of Rothschild in 1953 with some preliminary experiments with a photographic method with the intention to study sperm movement. In spite of some reasonably good photographs it was abandoned, partly because of technical difficulties in his laboratory and partly because of the complication of a large number of dead, non-progressing spermatozoa in most ejaculates. Rothschild used photographic plates and fixed exposures ranging from 1 - 1/500th of a second over active sea-urchin spermatozoa with a dark field illumination. He then measured the tracks created by the movement of the head to arrive at the distance travelled by the cells in the periods fixed by known time-exposure.

Most of the earlier work in this field was done in animals since with the introduction of the ability to store semen, the estimation of potential fertility of a particular seminal sample became an important commercial factor.

In 1959, Gassner criticised these photographic methods as not being able to distinguish between the varying degrees of

quality of motility. Botella (1956), Dott (1953), Gray (1955), Rothschild (1949) had used migration techniques in vitro. The method of Baker., Salisbury & Vandemark in 1957 was unsatisfactory for human semen because it assumes that the spermatozoa travel in a straight line, which is by no means true of human spermatozoa in a counting chamber. They also experienced similar problems to Rothschild's photographic techniques, due to the large number of dead, non-progressing spermatozoa.

Clare Harvey (1960) was one of the first workers who suggested that speed or velocity of human spermatozoa could be of relevance in clinical practice. She devised a technique for measuring sperm velocity by visualising and timing with a stop-watch the type of movement and speed at which spermatozoa entered and left a particular magnified area. The manner of movement of each spermatozoon was described into a tape recorder and was consequently a somewhat ponderous technique. The time taken for individual assessment was apparently no more than 7 minutes, though the transcribing of the tape took a rather longer time. In the earlier work the tracks were actually plotted on to paper, which was very time-consuming. Later the tracks were calculated directly from the transcribed recording and the velocity was calculated. However, the method had the main disadvantage - similar to that of the assessment of motility - that it was completely subjective. Also no allowance was made for the depth of the field in the counting chamber, which could permit some vertical movement.

The system can be criticised, as the selection of the sperm was

not truly random, since the human eye will always be attracted to the more motile sperm. She also found it necessary to dilute the seminal sample in order to be able to get a clear picture for proper assessment.

Her results in the light of later work were in fact reasonably accurate in spite of the rather simplistic method. Velocities between 30 - 68 μm/sec were detected from the human spermatozoa in a reasonably good sample. In contrast, however, she concluded that the actual dilution reduced sperm velocity, which is in strict contrast to the work of Makler (1979a & b) (to be discussed later). Further interesting data from this particular experimental work showed that the velocity of human spermatozoa was susceptible to changes in pH.

In discussion of her results in relation to fertility, which were correlated to a fertility index of her own devising, Harvey (1953) concluded that in the more fertile samples, the average speed tends to be higher than that of lower potential fertility. This led her to suggest that perhaps the speed of the spermatozoa may be an important factor in the fertility of samples which on the basis of density, motility and sperm morphology, appeared very similar. Here was the first example of sperm velocity being related to clinical practice.

In a paper entitled: "The Measurement of Human Spermatozoan Motility" John Janick (1970) measured sperm velocity and correlated it with the clinical parameter first suggested by Macleod & Gold (1951)

grading progression of human spermatozoa on a 1:4 basis. He pursued the methodology of Rothschild which had already been modified by Rikmenspoel (1957). He did this in the belief that the measurement of velocity in a very short time interval eliminates the possibility of deviations in forward progression of the cells. Again, Janick found it necessary to dilute in order to leave at least 10 - 15 motile cells in the photographic field. His diluent contained 15 per cent citrate and egg yolk. All seminal samples were reduced to the same sperm density. Although no actual data was presented, trial experiments with the diluent apparently did not show any appreciable effects upon motility and therefore did not affect the photographic measurements. He used a microscope adapted for dry dark field with a x 10 objective and a x 16 eyepiece fitted with a camera adaptor.

The methodology was simple. One drop of diluted semen was placed under a round cover slip and 10 fields which contained 10 - 15 active cells, photographed, the exposures being of the length of 1 second. The film used was Kodak 135 ASA. The resulting negatives were enlarged onto an 8 x 11 white tracing paper and the tracks of the spermatozoa were copied or traced by hand. In this work, as with Rothschild and Rikmenspoel, they were actually giving a photographic impression of the forward progression of the <u>head</u> of the spermatozoon which left a black track on a negative in the dark field illumination. The tracks were then measured in millimetres and the average velocity

computed in microns/second. Initially all this work was done at 20°C, but he later completed a small series at 37°C. The findings of the actual sperm velocity in microns/second was in relative agreement with Harvey with a range of from 15 to 55 microns/sec. Interestingly, when correlating his range of velocities with the grades of progression the "cut-off" between ++ and +++ was around 30 microns/sec. Janick also showed variation with temperature and that between room temperature and 37°C there is a distinct increase in sperm velocity. He concluded that this was a good technique for measuring the velocity of human spermatozoa in seminal plasma and that the velocity readings compared well with the subjective readings of motility. He also concluded that an acceleration in velocity is found when the environmental temperature of spermatozoa is elevated at 37°C.

In 1971 Bartak described a method for evaluating sperm velocity based on the direct visualisation of spermatozoa in a counting chamber using a stop-watch. He claimed that it was possible to calculate the average velocity of motile spermatozoa by knowing the sperm density count in millions/ml and the amount of time it took for 100 spermatozoa to traverse the counting chamber. The resulting calculation gave sperm velocity measurements at a level very much lower than all the other workers. He showed that there was a definite drop in velocity following ejaculation and that this drop was more marked in an oligospermic specimen as compared to a normospermic specimen.

He went on to state that the overwhelming majority of samples with sperm counts of under 20 million/ml have insufficient velocity. However, at this point in his discussion, he did not think that this particular way of measuring sperm velocity would be a substitute for the assessment of percentage motility. The work did underline the rapid drop in activity in ejaculates with poor seminal parameters.

Two further methods were reported by Sokoloski (1977) and Makler (1979a). The former stressed the limitation in measuring sperm motility as a clinical parameter. He accurately pin-pointed two aspects of sperm motility. One is the mathematical description of the flagellar wave which gives the sperm some of its characteristic swimming pattern and velocity. The second is the relationship between the motility of a given sample of sperm cells and their fertilising capacity. He stated:- "It seems reasonable to suppose that sperm velocity containing a high fraction of vigorous cells are the best candidates for high fertilising capacity". He went on to say that all this could be established, but it was necessary to produce a methodology for measuring average velocity in order to provide quantitative data for eventual statistical correlation.

The basis of his method was two-fold: firstly, that a population of sperm moving into a medium in an optical path will produce a time-dependent increase in absorbence. This

increase in turbidity is proportional to the average rate of movement in the cell population and to the concentration of cells. Secondly, that the spermatozoa with the highest motility and with the greatest directional persistence will be the first to swim upward into the optical path from the bottom of the "couvette" and therefore will cause an increase in turbidity.

This turbidity was then measured by spectrophotometer with a tungsten light-source and was recorded on a strip-chart recorder.

He recorded at 30 minutes following ejaculation with a pH of 7.6 and at a temperature of $37^{o}C$. From the recordings he was able to calculate an average sperm velocity. The advantage of this method is that it is rapid and requires only a single spectrophotometer. It also gives an average velocity of a much greater sample than used in microscopic techniques. He claimed that it would be possible by using this method to correlate motility parameters and fertilising capacity of patients. However, since then, no further information of its use as a clinical parameter has been published.

The second methodology by Makler was, interestingly, very similar to our own. He also used time-delay photography. Rather than using a continuous fixed period of exposure, he used

a new multiple-exposure technique consisting of a time-delay of 1 second - the film being illuminated by 6 stroboscopically induced light pulses. Again it was done under phase contrast microscopy. Like the previous work of Janick he worked out his average sperm velocity measuring the distance covered by the head of the spermatozoon. The actual velocity calculations were done manually and also using a digitalizing computer system. He went on to show in this paper that both sperm motility and velocity change with ageing of the specimen. The progressive decrease of motility in the first 24 hours is already an established fact. What was most interesting was that, in complete contrast to Harvey, he showed that there was in fact an increase in velocity up to 4 hours following ejaculation. He explained this by the continuance of metabolic and enzymic processes which are responsible for velocity.

This information was also in contrast to that of Bartak (1971) which showed a reduction in velocity one hour after ejaculation. It should be noted that with this method, there was no form of dilution and Makler feels this is an important advantage of his particular method.

At the same time he produced further information (1979c) in which sperm velocity and the percentage of motility in 100 normospermic specimens were again analysed by his method of multiple-exposure photography. The purpose of this study was to determine how three most important characteristics of semen -

namely sperm concentration, motility and morphology, were interrelated and he showed that no correlation exists between sperm count and the percentage of motility or velocity. There was moderate correlation between spermatozoa velocity and percentage of motility, but motility is negatively correlated with abnormal forms - and suggested that this might throw light on the influence of spermatozoan morphology and the quality of semen.

Comparing his figures for sperm velocity in microns/sec to other workers in this field, already discussed, he showed that his value of velocity was very close to that described by Harvey and Janick; but in contradiction to Bartak's data which gave an average sperm velocity of normospermic samples of up to 100 microns/sec. However, he joined in disagreement with the data of Sokoloski whose turbidity method gave even higher results.

Makler went on to discuss the effect of dilution on seminal samples and the possible effect on velocity measurements. He claimed that progressive dilution will affect seminal viscocity and enable sperm to increase their speed. He felt that this method which had shown direct measurements on undiluted specimens was better than those involving dilution. The correlation he found between sperm concentration and percentage of motility was similar to that found by Rehan (1975). However, the low correlation between motility and velocity led him to suggest that these two were two specific independent parameters.

Finally, he suggested a new index of motility, combining the data of spermatozoal velocity and the percentage of motility. This would give a true average speed of all spermatozoa in the specimen and was suggested as an important parameter in routine semen quality evaluation.

From the review of the literature, it was felt that the method for assessing sperm velocity using microscopically taken photographs under a time-delay mechanism still had potential for further development.

CHAPTER II

MATERIALS AND METHODS

THE DEVELOPMENT OF TIME LAPSE PHOTOGRAPHY (TLP) AS A METHOD FOR ASSESSING SPERM VELOCITY

Introduction:

The original concept of time-lapse photography as a method of producing a permanent record of spermatozoal movement has already been discussed in Chapter I. The first priority was to produce negatives which would allow for visual measurement in various parameters required to calculate sperm velocity. Previous work was confined to the bull spermatozoon which has a much greater velocity than that of the human. The various experiments carried out therefore, are enumerated below, and were necessary in order to adapt this methodology to the characteristics of the human spermatozoon. The selection of the correct conditions in order to produce an easily evaluated picture was initially purely visible and thus subjective. Obviously until such time as the right subjective result could be achieved, the calculation of sperm velocity was impossible.

Selection of Equipment:

The basic unit was a Vickers M41 Photoplan (see Fig. 2.1) microscope to which was fitted a 35 mm camera which incorporated an automatic wind-on facility. Further equipment included a Vickers power unit (100 watt) into which a variation of lamp voltage was built. A Vickers photometer timer (J37) was also incorporated. The timer part of this equipment gave variable exposures from 0.1 second through graduated steps to 8 seconds. Variation of lamp voltage was monitored by the photometer.

The microscope was set up with negative-phase in order to produce a positive "negative" for evaluation. A x 20 negative-phase objective was used with a x 10 eyepiece incorporating a graticule for sharp focusing for photography. A standard green filter at the light source was used in order to improve contrast, as advised by the makers. Control of slide temperature at the time of photography was achieved with the use of a Microtec Warm Stage which gave a variation of reading from 15°C to 45°C. In view of the necessity of the warm stage, a long-range condenser had to be fitted to the M41 Photoplan Microscope.

Figure 2.1: Vickers M41 Photoplan Microscope With Light Source, Phototimer And "Microtec" Warm Stage.

METHOD OF CALCULATING SPERM VELOCITY

One outstanding problem was the method of quick calculation of the velocity, from the photographic fields. Ojakian & Katz (1973) and later Katz & Dott (1975) studying full sperm velocity devised a formula for the calculation of this parameter from photomicrographic prints produced by the tracks of spermatozoa over a 1 second exposure time. They showed that the velocity could be calculated from the following formula:

$$\text{Velocity} = \frac{\pi}{2} \times \frac{N}{Ltn}$$

where N = number of motile cells crossing the diagonals, L = length of the diagonals in micrometers, t = the time exposure (2 seconds) and n = the photodensity (i.e. field area in square micrometers divided by the number of motile cells in that field.)

It should now be possible to take microscopic pictures of spermatozoal activity and by calculating the number of tracks that crossed a known length of diagonal, be able to calculate the average velocity of the motile spermatozoa.

In all previous experiments the investigators had concentrated on the tracks caused by the movement of the head of the spermatozoon. In this series of experiments it was felt better to concentrate on the track of the spermatozoon since it was this part of the sperm which actually produced the forward movement. It was further felt that under negative-phase light the actual wave of the tail would reflect

the light in such a way as to produce a permanent photographic record. These trails of what could best be described as flashes of the sperm tail could then be converted into a sperm velocity measurement in the manner described above.

To establish the correct strength of light-source in relation to the duration of exposure time (time-lapse).

Patients and Methods:

Four volunteers were recruited, and samples were obtained by masturbation into a sterile container. Each sample was kept at 37°C in a warm bath for 30 minutes to allow for liquefaction. The Microtec warm stage was set at 37°C throughout photography. For each specimen, the following procedure was carried out: Photographs were taken at the following lamp voltage readings: (volts 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12). This process was repeated at the following time exposures:
0.5 sec, 1 sec, 2 sec, 4 sec.
The film used was a 125 ASA (FP4) variety.
Following photography the films were developed by a routine standard commercial method.

Results:

Visual determination of the films showed that a lamp voltage of 7 with a time-lapse of 2 seconds gave the clearest and most easily discernible picture of spermatozoal activity. This interestingly, confirms the already known knowledge that bull spermatozoa have twice the velocity of human ones; whereas in estimation of bull sperm velocity, 1 second delay is the best measurement, in humans 2 seconds

appears to be the ideal. The importance of the duration of exposure time in the calculation of sperm velocity will be discussed later.

Variation of the film speed (in ASA) in order to produce the best quality of photographic field.

Patients and Methods:

Four volunteers were recruited as before and samples were produced as already described. At exactly half an hour following ejaculation, photographs were taken with a time-lapse of 2 seconds and at a lamp voltage of 7 (see previous experiment). Photographs of the samples were taken using films with the following ASA: 30, 60, 125, 200, 400.

Results:

From a visual determination of film, projected on to a square piece of white paper by a Durst enlarger, showed that the films taken with 125 ASA (FP4) gave the clearest picture of sperm trails.

It was therefore decided to use ASA 125 FP4 film throughout the remaining part of the investigations.

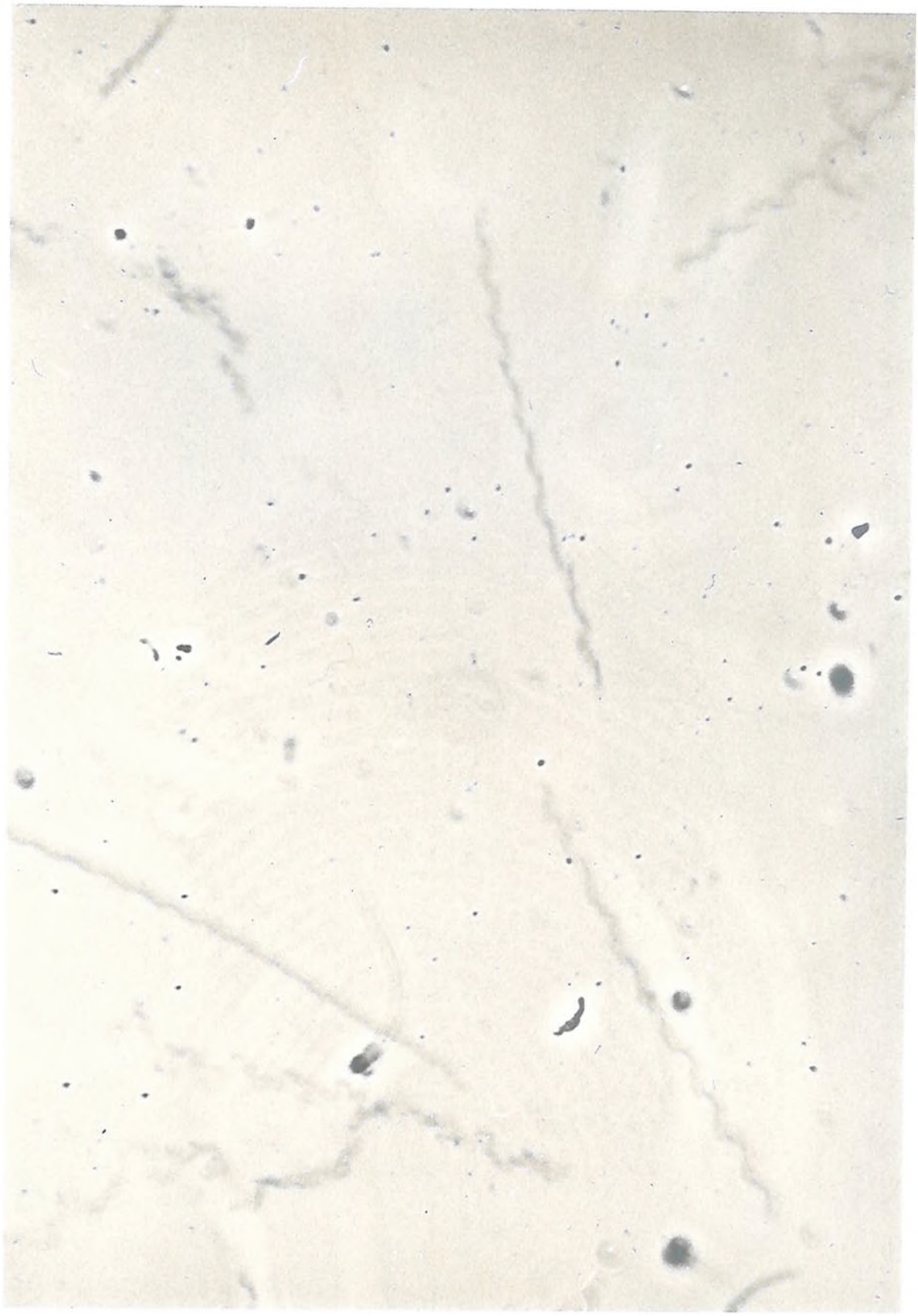


Figure 2.2: Typical Photographic Field of TLP Exhibiting Good Sperm Velocity.

Selection of Suitable Sperm Diluent.

From the above series of experiments and from the information of other investigators (see Review of Literature) it became apparent that a diluent was required. Too few or too many sperm trails made evaluation inaccurate and it was soon realised that it was necessary to get a suitable number of motile spermatozoa in the photographic field in order to give a reliable result. Secondly, there is a great variation in seminal debris from one sample to another, which in some cases made sperm trail evaluation almost impossible.

On the basis that in the in vivo situation the motile spermatozoa soon leave the seminal fluid environment and in a short space of time are found in both the uterus and Fallopian tube, it was felt that a diluent as similar as possible to the in vivo situation was required. The assessment of the sperm velocity would therefore take place in a situation which was similar to that which would occur normally in the body. From the work of Lopata et al (1976) who extracted human tubal fluid at the time of laparoscopy and analysed its constituents, a solution was made up similar to the strength of the constituents shown in his paper (see Table 2.1).

From previous experiments it had been noticed that in order to produce a suitable film field for evaluation, it was necessary to have 10 - 20 motile spermatozoa within the

photographic field:

This produced two main problems -

Would the diluent affect sperm velocity either

(1) by producing a change in viscosity, thus changing the resistance the motile sperm had to overcome;

(2) by the effect of the constituents in the diluent.

The constituents of the artificial tubal fluid were as follows:

Table 2.1 : Constituents of Diluent (Lopata et al, 1976).

Na Cl	5.3 g
K Cl	0.35 g
$K H_2 PO_4$	0.05 g
$Na HCO_3$	3.0 g
$CaCl_2 2H_2O$	0.3 g
$MgSO_4 7H_2O$	0.05 g
Sodium pyruvate	0.36 g
Sodium lactate	4 ml of 60% soln
Glucose	1.0 g
Human Albumin	2 ml of 20% soln
HCL (1N)	1 ml
Crystamycin	44 mg

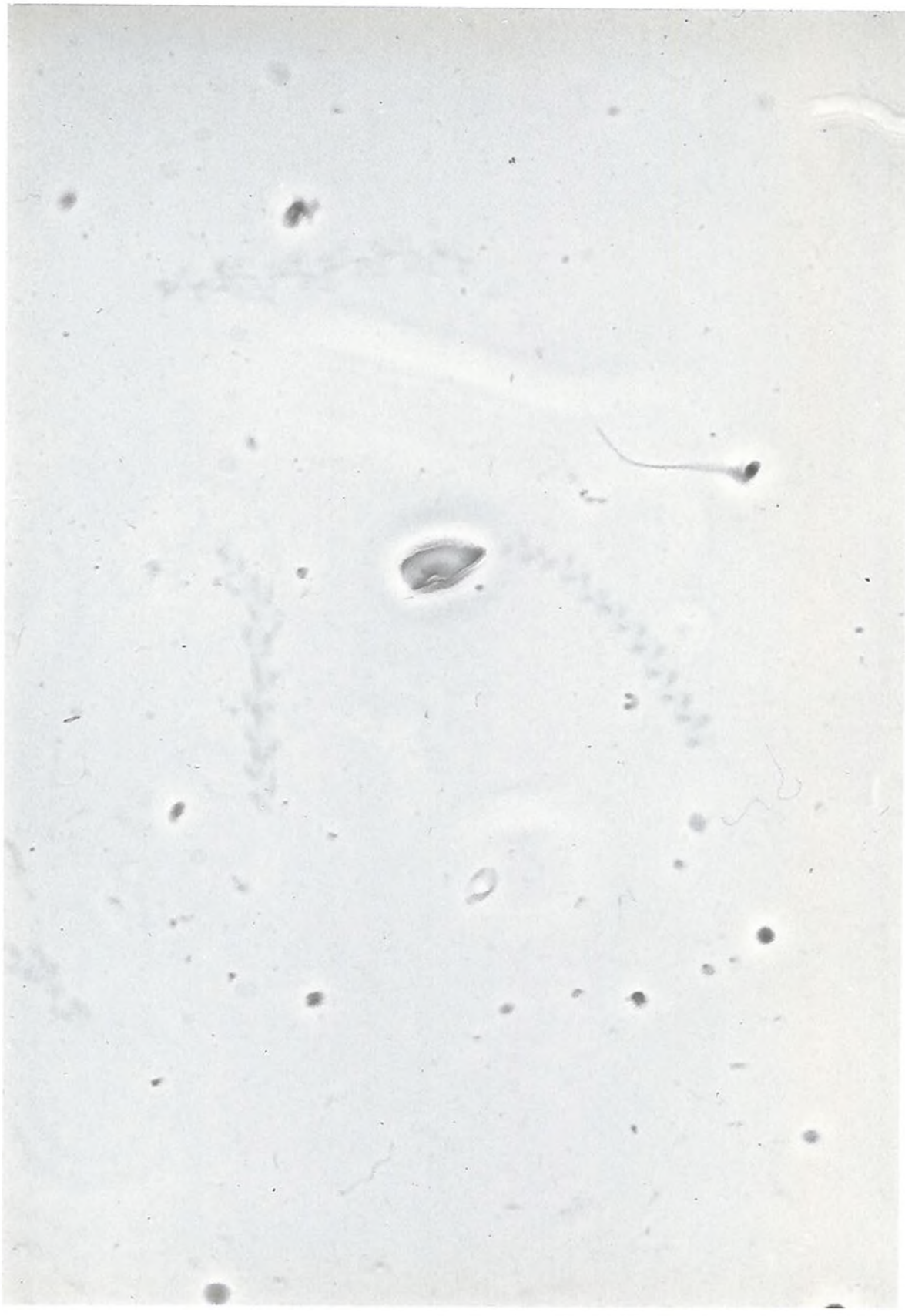


Figure 2.3: Typical Photographic Field of TLP Exhibiting Low Sperm Velocity.

The effect of diluent (Lopata et al) on sperm velocity as measured by time-lapse photography (TLP).

Introduction:

The progressive increase in dilution in order to provide an ideal field for photography may have an effect on sperm activity due to the constituents of the sperm diluent (Lopata et al, 1976) already described. The previous work by Harvey (1959) showed that dilution caused a reduction in velocity. However, Janick & Macleod (1970) were also using a photographic method (see Review of Literature) and found it necessary to dilute those samples with a higher density. They used a diluent containing 15% citric acid and egg yolk. They found that there was no effect on sperm velocity as measured by their method. However, it should be noted that this diluent was not added to all of their specimens. In contrast, Makler (1979, 1979b) showed a progressive increase in sperm velocity when comparing diluted samples to undiluted. However, it should be stressed that there was no significant difference between the three strengths of dilutions incorporated in his paper.

In view of this contradiction of information, it was decided to test the effect of various strengths of the diluent and its effect on sperm velocity as measured by TLP.

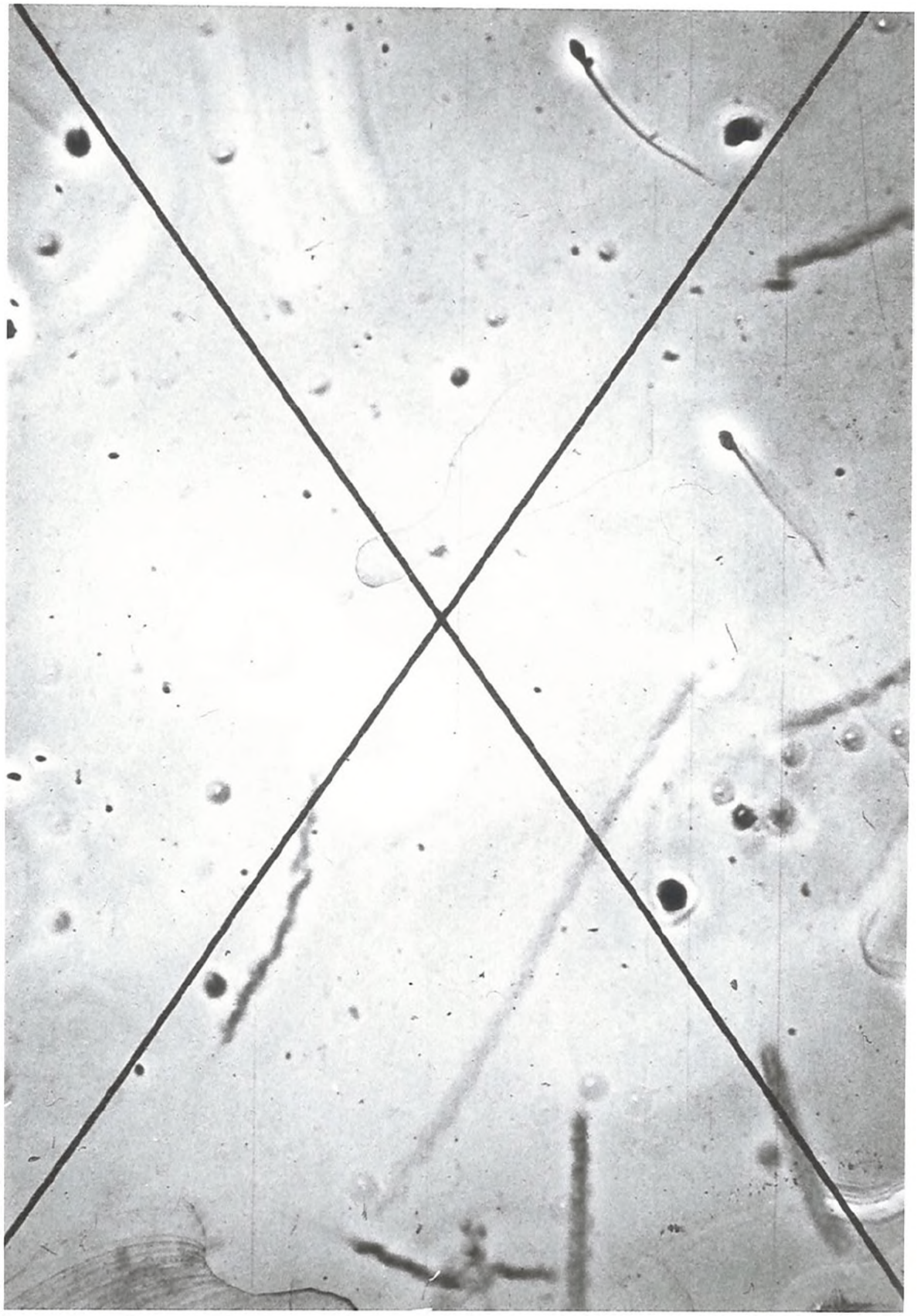


Figure 2.4: Photographic Field (TLP) With Diagonals In Situ.

Methods:

As already stated, the Vickers M41 Photoplan Microscope and phototimer was used with a fixed exposure of 2 seconds and a constant light source (see Figure 2.1). A x 20 negative-phase objective was used with a x 10 eyepiece incorporating a graticule for sharp focusing and a long-range condenser. The temperature was kept constant at 37°C using a Microtect 'warm' stage. A film speed of 125 ASA (FP4) was used. Twelve individually focused films were photographed randomly at each dilution. The various strengths of dilutions tested were as follows:

Undiluted, 1:1, 1:2, 1:3 and if density allowed it, 1:4. The films were developed in a routine manner and assessed with the use of a Durst enlarger. The velocity was calculated using the formula as already stated.

A full seminal analysis was carried out. Percentage of motility was measured from a visual assessment of the photographic fields. The number of non-motile and motile spermatozoa were calculated. In the case of number of motile spermatozoa, a correction was carried out on the basis of the work of Ojakian & Katz (1973) who showed that when comparing their method to actual video micrographs, it was necessary to divide by 2 all those tracks crossing the edges of the photographs in order to get comparitive motility figures.

Having therefore carried out this correction of the percentage motility as calculated in relationship to the total number of non-motile sperm in the twelve fields assessed, all

the above information, including that of seminal analysis, was fed into a computer programme kindly provided by Dr. Martin Harman of the Primary Medical Care Unit, Southampton University. This programme also carried out the statistical analysis of all the data presented in this thesis.

For the purpose of the above experiment, only the sperm velocity, standard deviation, sperm density, percentage of motility and volume are reported.

Patients:

Five healthy young volunteers were recruited and the results of their semen analysis are shown in Table 2.2.

Table 2.2: Seminal Analysis (n = 5)

Patient No.	Sperm Density	% Motility	Vol (ml)
1*	58×10^6	50%	2.2
2**	125×10^6	75%	3.5
3**	60×10^6	70%	2.5
4**	32×10^6	65%	3.8
5***	45×10^6	40%	7.8

* Unproven fertility

** Fertile

*** Infertile

Results:

Table 2.3: Variation of Sperm Velocity (μm/sec) in Undiluted and Progressively Diluted Samples (n = 5).

		Sperm Velocity (μm/sec)				
		Undiluted:	1:1	1:2	1:3	1:4
1*	Mean	29.4	32.35	30.7	29.6	30.7
	SD	5.4	7.2	6.7	5.3	9.4
2**	Mean	36.0	32.0[a]	32.0[a]	33.7	30.9[a]
	SD	3.9	3.9	4.1	5.2	5.7
3**	Mean	31.2	28.8	26.7	27.8	-
	SD	5.5	3.2	4.6	4.4	-
4**	Mean	26.8	26.6	26.80	29.2	-
	SD	4.3	4.7	4.95	6.34	
5***	Mean	22.9	26.27	24.5	24.4	-
	SD	6.6	7.02	5.7	6.1	-

* Unproven fertility
** Fertile
*** Infertile

a = significant difference ($P < 0.05$) to indiluted.

When considering the diluted samples and comparing the various strengths from 1:1 to 1:4, there was no significant difference between any of the various dilutions in all five patients studied. They include both fertile and infertile patients. In comparison of the undiluted samples to diluted, the only significant difference was in Patient 2, as indicated in Table 2.3.

Conclusions:

It appears from the above data that the various strengths of dilutions of this particular diluent has no effect on the sperm velocity, though there does seem to be some difference between diluted samples and undiluted samples when looking at Patient 2. This information therefore appears to agree with Janick and Macleod (1970) and to be in disagreement with the conslusions of Makler (1979b). However, it must be pointed out that in that paper there was also no significant difference between the various strengths of dilutions. The data therefore does not agree with Makler's assertion that increasing dilutions will have a stimulating effect upon sperm velocity. Makler's other claim that dilution will affect the sperm velocity due to jostling of the sperm (Tampion & Gibbons, 1963) also does not appear to be apparent. However, in that paper, the jostling effect was only noticed in sperm densities over 20 million/ml, since in this experiment all samples were diluted to below 20 million may explain the apparent difference in sperm velocity in undiluted and diluted samples of Patient 2.

It could also be argued that it is unphysiological to measure sperm velocity in seminal fluid, since in the in vivo situation the spermatozoa only spend a very short time in contact with it.

The final conclusion of the above experiment was that progressive dilutions with this diluent does not have an effect on sperm velocity as measured by TLP.

To test the viscosity of sperm diluent on sperm velocity as measured by time-lapse photography (TLP).

Introduction:

Having established that the degree of dilution with one diluent does not affect sperm velocity, it was now necessary to compare two different sperm diluents with contrasting viscosity. It was therefore decided to test the difference between the addition of the now standard sperm diluent - as already described - against a diluent containing 10% glycerol.

Patients:

A healthy young volunteer was recruited whose standard seminal analysis was as follows (Eliasson, 1971):

Table 2.4 :

Density	:	100 million/ml
% Dead	:	10%
Volume	:	5 ml
% abnormal forms	:	25%
% motility	:	65%

Methods:

To the one ejaculate, the similar volume of both diluents under investigation was added in order to obtain the necessary number of motile spermatozoa in the photographic field.

This experiment was repeated on five occasions. Calculation of sperm velocity was carried out using the same computer programme and the Table below gives a summary of the results.

Results:

Table 2.5 : Sperm Velocity (μm/sec) in Two Different Sperm Diluents of Varying Viscocity.

	Artificial Tubal Fluid (Lopata)	Glycerol
1	39.42 ± 7.57	21.27 ± 6.23
2	42.43 ± 9.79	24.11 ± 6.92
3	37.97 ± 6.35	26.60 ± 8.86
4	38.28 ± 7.56	23.55 ± 7.44
5	38.34 ± 8.21	22.75 ± 4.84

Values are means ± 1 standard deviation.

Table 2.5 shows the effect of viscosity on sperm velocity, of one ejaculate measured five times.

From Table 2.5 there was a significant difference between the group diluted with tubal fluid and that with the diluent containing glycerol and this difference was highly significant ($p = 0.0009$).

Conclusions:

It was concluded from this experiment that a large increase in viscosity had a definite effect on the sperm velocity. This

would be expected, since the sperm velocity must be related to the resistance that the spermatozoon has to overcome in order to proceed forwards. However, it should be stressed that in the previous experiment where increasing concentrations of one diluent were used, no significant difference was found in sperm velocity. Therefore, changes in sperm velocity will only occur when the viscosity of the diluent is markedly different.

From the above data it was felt that the specimens could have different dilutions without affecting the measurements of sperm velocity and consequently throughout the remaining part of the investigation, the specimen was diluted according to density.

To test the repeatability in the measurement of sperm velocity by time-lapse photography (TLP).

Having established that it was possible to calculate sperm velocity using this method and that dilution was necessary for good photographic results and did not affect the final calculations, it was now necessary to test the repeatability of this method.

Methods:

A similar method was used as to the last experiment and again a constant temperature of 37°C was maintained. A time-lapse of 2 seconds was used and 125 ASA FP4 film was utilised. A healthy young volunteer was recruited to participate in the study and was required to abstain from sexual activity for three days prior to sample testing. Again the specimen was obtained by masturbation and assessed within thirty minutes. Each sample was split into three aliquots and slides were made up from the three samples with similar dilutions. On each sample, seven randomly photographed films were taken and assessed by the standard method. Sperm velocity was calculated from the formula already given.

Results:

Table 2.6 : Variation of Sperm Velocity Between Samples from One Ejaculate.

	Sample 1	Sample 2	Sample 3
Mean Velocity (μm/sec)	36.68	37.91	35.38
SD	4.29	10.23	8.54

There was no significant difference between the three samples and the coefficient of variation was 3.43%. This was highly acceptable as far as repeatability was concerned. However, it was decided to repeat this experiment in further ejaculates and different sperm diluents.

To re-test the repeatability of the measurement of sperm velocity by time-lapse photography in different dilutions.

The purpose of this experiment was to confirm the data achieved in the previous experiment and to see also if there was a different standard of repeatability according to the diluent used.

Methods:

Again, a healthy young volunteer was recruited and exactly the same procedure was carried out as in the previous experiment. The sample was split into ten aliquots. In the first five, similar dilutions of human tubal fluid substitute was added and in the final five a diluent containing 20% glycerol. Previous experiments have already shown the difference in sperm velocity in these two diluents.

Results:

In Table 2.7 there was no significant difference in the five samples when measuring sperm velocity; the coefficient of variation in this case was 4.65%.

Table 2.7 : Variation of Sperm Velocity between Samples from One Ejaculate (Tubal Fluid Diluent).

	Sample: I	II	III	IV	V
Mean Velocity	39.42	42.43	37.97	38.28	38.34
SD ±	7.5	9.9	6.3	7.5	8.2

Table 2.8 : Variation of Sperm Velocity between Samples from One Ejaculate (20% Glycerol Diluent).

	Sample: I	II	III	IV	V
Mean Velocity	21.29	24.11	26.60	23.55	22.75
SD ±	6.2	6.9	8.8	7.4	4.8

In Table 2.8was a markedly different diluent and again there was no significant difference between the sperm velocities in the five samples, but the coefficient of variation was slightly increased at 8.25%.

Summary:

The repeatability in the measurement of sperm velocity by time-lapse photography when using the diluent made up similar to human tubal fluid gives a coefficient of variation in the region of 3 - 4.65%.

To determine the effect of temperature on the velocity of human spermatozoa as measured by time-lapse photography.

Introduction:

Having established the methodology of measuring sperm velocity from time-lapse photography and having already shown the method to have a reasonable repeatability, it was decided to test the effect of temperature on the velocity of human spermatozoa.

Patients and Methods:

Four healthy young volunteers participated in this study as before. Again, they were required to abstain from sexual activity for three days prior to sample testing. Samples were produced in the normal manner and suitable dilution took place and seven randomly focused films were taken at each temperature. The temperature was varied by using the Microtec 'warm' stage from 37°C to 40°C with increments of 3°C. Again a standard seminal analysis was carried out according to Eliasson (1971).

Results:

Table 2.9: Sperm Velocity Versus Temperature.

	Velocity[a] (μm/sec)			
	Sample 1	Sample 2	Sample 3	Sample 4
25°C	26.01 ± 6.73	24.10 ± 8.4	25.10 ± 6.08	28.26 ± 5.45
28°C	35.69 ± 6.98	43.34 ± 9.12	42.44 ± 11.78	31.32 ± 6.69
31°C	37.86 ± 10.42	44.53 ± 11.62	31.79 ± 14.36	37.97 ± 4.93
34°C	37.72 ± 9.84	46.85 ± 10.4	35.74 ± 13.07	45.89 ± 5.41
37°C	43.64 ± 9.84	48.36 ± 9.19	48.63 ± 11.17	46.67 ± 5.77
40°C	44.89 ± 19.65	51.84 ± 13.69	51.68 ± 5.62	44.44 ± 10.94

[a] Values are means ± standard deviation.

Looking at Table 2.9 and Figure 2.5 showing sperm velocity against temperature, it can be seen that in all four samples there was a gradual increase in sperm velocity when increasing the temperature. A statistical difference at the 5% level in all four samples between sperm velocity and 25°C and 40°C was seen. For this reason it was decided to regulate all future measurements at 37°C.

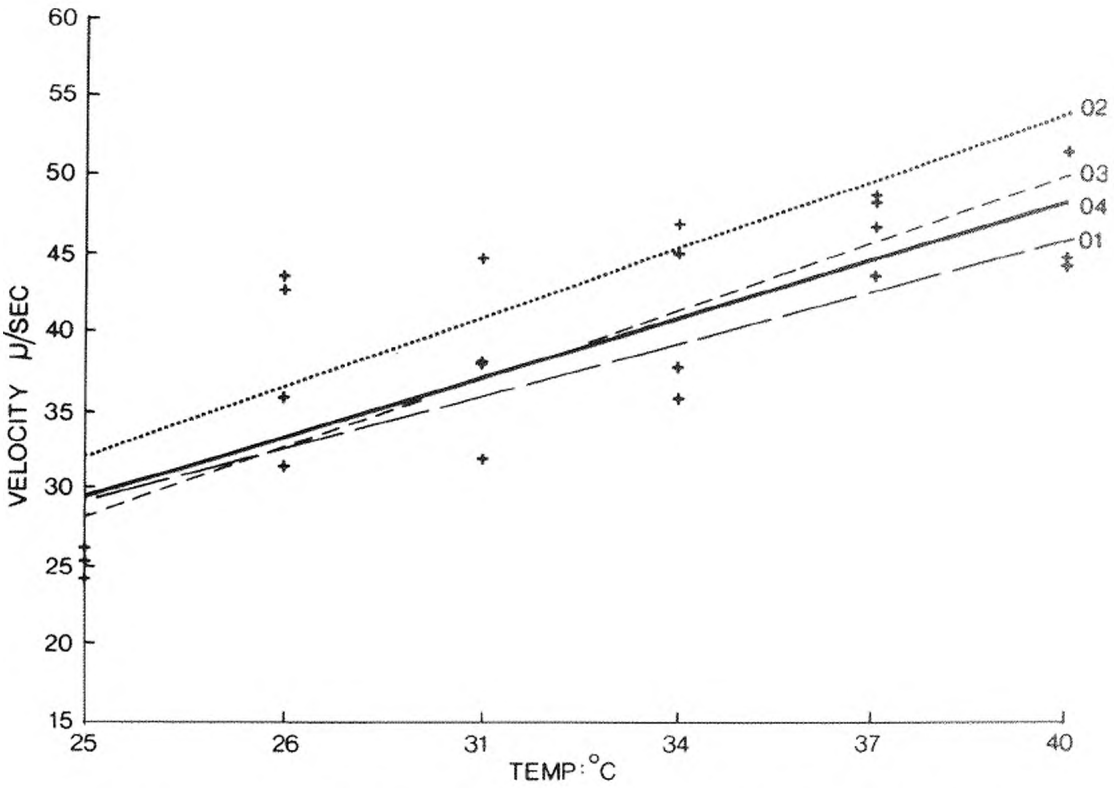


Figure २.५ showing effect of Temperature - (°C) against Sperm Velocity (μ/sec)

To determine the effect of pH of the diluent on sperm velocity.

Introduction:

In setting out his methods of standard seminal analysis, Eliasson (1971) suggested that 7.6 was the optimum pH to use in evaluating the various parameters already discussed. With this in mind, it was decided to test the measurement of sperm velocity at different pH's to determine another possible variable.

Patients and Methods:

A young volunteer with normal other seminal parameters provided an ejaculate in the above-described manner. The diluent was made up as before and the pH was adjusted to give the following pH's: 7.3, 7.5, 7.7, 7.9, 8.1 and 8.3. The same degree of dilution was added to each ejaculate at the different pH's. Twelve fields randomly focused with all other parameters staying as before; the temperature was kept at 37°C throughout. The films were assessed in the usual manner and calculation of average sperm velocity was made.

Results:

The results showing the effect of the pH of the diluent against sperm velocity are shown in Table 2.10 and also in Figure 2.6. There is a progressive rise in the sperm velocity of a single ejaculate to 8.1 where it then falls to 7.3. Between the levels

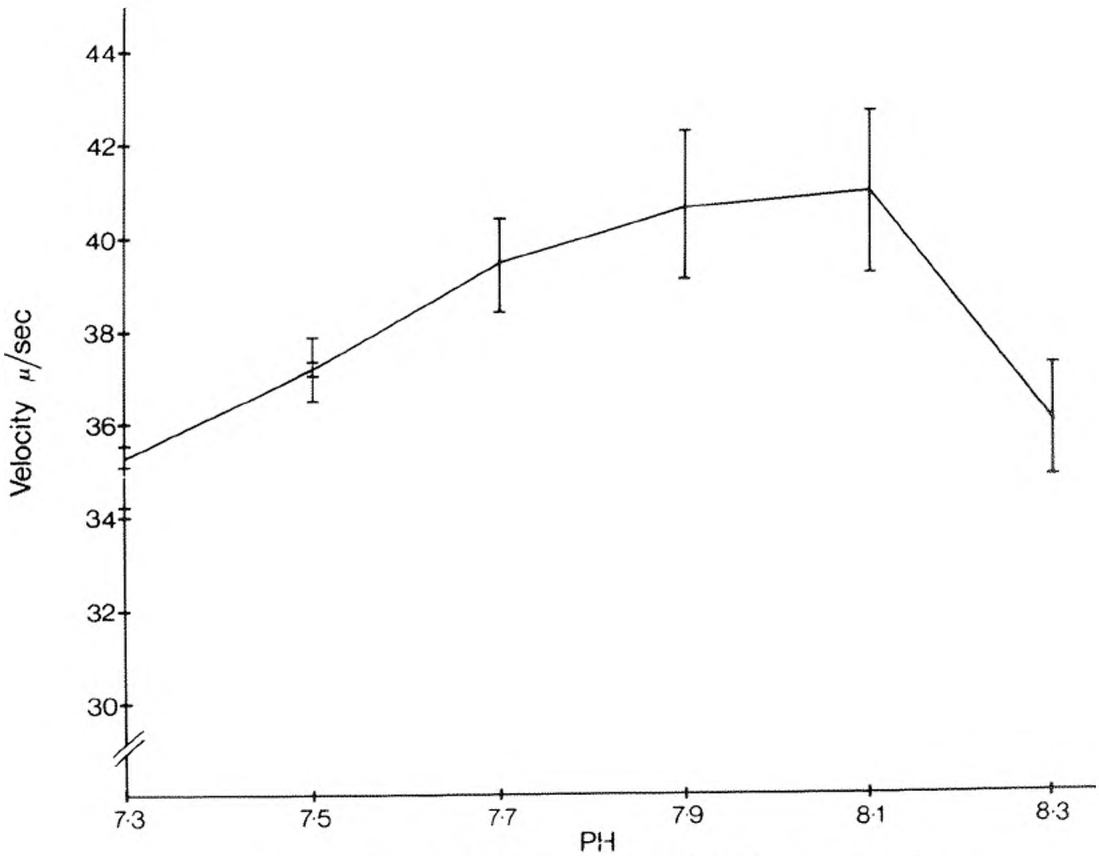


Figure 2.6 showing Sperm Velocity ± SD (μ/sec) against PH

of pH 7.7 and 8.1 there is no significant difference ($p < 0.05$). Our selection of pH 7.6 was in the optimum range and was used throughout all remaining experiments using this methodology.

Table 2.10: Sperm Velocity (μm/sec) against pH.

	pH					
	7.3	7.5	7.7	7.9	8.1	8.3
Mean Velocity	35.35	37.21	38.73	40.83	41.11	36.14
SD	7.53	4.05	5.89	10.03	12.80	9.33

Conclusions:

The data found in this experiment shows a steady increase in sperm velocity up to 8.1 and the rapid drop is very suggestive of an enzymic curve which would be appropriate in view of the knowledge on enzymic action in the production of sperm motility. The changes noted in this experiment were not significant.

Overall Conclusions in the Methodology of Time-Lapse Photography (TLP)

The preceding experiments in the use of TLP have established a reliable method of assessing human sperm velocity in diluent similar to human tubal fluid, with a coefficient of variation of 4 - 8%. The variables include time-delay (2 seconds) and lamp voltage (7 volts), temperature (37°C), film speed (125 ASA), pH (7.6), the number of fields counted (12) and the viscosity of human sperm diluent.

The above figures for variables will be used throughout the remaining useage of TLP in this Thesis and will therefore become the standard procedure.

STANDARD SEMINAL ANALYSIS:

Standard seminal analysis carried out throughout this Thesis follows the lines of that set out by Eliasson in 1971.

(a) Sperm Count/Sperm Density:

Sperm density evaluation was carried out following photography. All samples were by this time liquefied. The specimen was diluted to 1:20 dilution in a diluent containing 4% formaldehyde and 5 g/100 ml $NaH\,CO_3$. This was mixed well, placed in the improved Neubauer counting chamber and visualised under a x 400 magnification. The sperm in 10 triple ruled squares were counted, 5 from each half of the chamber counted diagonally. In a 1:20 dilution, the total number of sperm counted divided by 2 is equal to the number of sperm in millions/ml, in the specimen. If the density was visualised to be low initially, then a lower dilution was carried out accordingly and the calculation was adjusted. The above is a standard method of calculating sperm density and is known to have an individual error of up to 20%.

(b) Live/Dead Stain:

The percentage number of dead sperm were calculated by the

following method:

One drop of semen using a Pasteur pipette, after thorough mixing was added to four drops of the stain containing the following constituents: Eosin 0.67 g;

Nigrosin 10 g in 100 mls of sterile water.

After standing for one minute, the specimen was smeared on to a slide and allowed to dry. The specimen was visualised under a x 400 magnification. The dead sperm were identified since they took up the stain. 100 spermatozoa were counted and the percentage of dead spermatozoa calculated.

(c) Morphology of Spermatozoa:

Three fully liquefied drops of semen were mixed with two drops of formaldehyde bicarbonate solution, the constituents of which are described in the measurement of sperm density. The solution was mixed thoroughly. A smear was then made on a clean slide and allowed to dry. The slide was then passed on to the Cytology Department, where standard Papanicolaou stain was carried out - as in a routine semen analysis.

Following staining the preparations were visualised under magnification by a technician providing this service in Southampton General Hospital and the percentage of abnormal forms was counted over 100 spermatozoa.

To avoid observer bias the same worker carried out the examinations of morphology, unaware of which group she was reporting on.

(d) Ejaculate Volume:

The volume was measured using a sterile glass measuring cylinder.

The sperm density, the percentage dead cells, the percentage abnormal forms and the specimen volume were fed into the programme computer and the following parameters were established:

Sperm density

Absolute number of sperm

Absolute number of live sperm

Absolute number of normal sperm

Specimen volume.

The remaining sample was centrifuged at 4000 revs per minute for 20 minutes. The seminal fluid was removed and frozen immediately to $-20^{o}C$ to await further analysis.

SERUM INVESTIGATIONS

(a) 17β Oestradiol:

17β oestradiol was measured by radioimmunoassay by Mr. F. Anthony with an antiserum approved by the Supra-regional Service. A pooled male plasma control was made up and used in each assay to evaluate the variation from assay to assay (inter-assay coefficient of variation). To incorporate the same variation in the fertile and infertile groups each assay run incorporated samples from both groups.

The assay employs the adjustment of pH of the plasma to 10.5 with a carbonate buffer and the extraction of the un-conjugated 17β oestradiol into diethyl ether. The diethyl ether is evaporated off and the 17β oestradiol re-dissolved in the assay buffer. Antisera and tracer are added and a solvent bank is also run at the same time. The radioactive tracer is tritiated 17β oestradiol obtained from the Radiochemical Centre at Amersham. The intra-assay coefficient of variation was 5% and the inter-assay coefficient of variation was 13% in a sample with a mean value of 152.14 pmols/l.

(b) Serum Prolactin:

Serum prolactins were kindly measured by Dr. Anne Evans in the Department of Chemical Pathology, Southampton University. She used a homologous radioimmunoassay using sheep antisera to

human prolactin kindly provided by Dr. Graham Groom, Tenovus Institute, Cardiff. The radioactive antigen was kindly donated by Dr. P. G. Lowry, Chemical Pathology, St. Bartholomew's Hospital. A laboratory standard was used which was calibrated to the first International reference preparation code no: 75/504 and this assay was part of a national quality-control service. The intra-assay coefficient of variation was 2.8% and the inter-assay coefficient of variation was 13.3%

(c) Serum FSH and LH:

Follicle stimulating hormone and luteinizing hormone were measured by Dr. Peter Woods, using a radioimmunoassay on the United Kingdom recommended scheme in the Department of Chemical Pathology, Southampton University. The antisera was kindly supplied by Professor W. Butt from the Womens Hospital, Birmingham. The radioactive tracer was supplied by the Chelsea Hospital for Women, London; the laboratory standard used was that of the Medical Research Council which for luteinizing hormone was code no: 68/40 and for follicle stimulating hormone, code no: 69/104.

Both assays were controlled by the use of quality control pools at four levels of test. The between-batch coefficient of variation ranged from 14.8% for LH level of 2.7 IU/1 to 5.2% at a level of 34.8 IU/1. For FSH the between-batch coefficient of variation ranged from 9.1% at a level of 2.2 IU/1 to 5.2% at a level of 25 IU/1. Both assays were assayed by the U.K. National External Quality Assessment Scheme run from Edinburgh and the

Department of Chemical Pathology, Southampton University, being one of the reference groups for these assays. Each assay batch contained specimens from both fertile and infertile patients, as described, with 17β Oestradiol. The intra-assay coeffient of variation was 5 - 10%.

(d) Dihydrotestosterone (DHT):

DHT was again kindly measured by radioimmunoassay, by Dr. Peter Woods in the Department of Chemical Pathology, Southampton University. He utilised a kit supplied by Amersham containing its own antisera and standards. The code number of the standards was no: TRK/600. The inter-assay coefficient of variation for an assay of a quality control pool with a mean DHT levels of 2.4, 1.8, 0.9 nmols/l were 7.4%, 8% and 12.4% respectively. As with FSH, LH and 17β oestradiol, each batch contained specimens from fertile and infertile patients.

Seminal Plasma.

Seminal Fructoseand Seminal Myoinositol:

These two constituents were measured by a semi-quantitative paper chromatographic method, as reported by Lewin (1978).

The method was as follows:

A set of samples (1 microlitre) of seminal fluid, was placed on the origin line, 2 cm from the bottom of a filter paper sheet (Whatmanno: 1, 23 cms x 28 cms) and was developed by the ascending technique at room temperature, for three hours, using the solvent system 2, 6 lutidine-isopropanol-water (60:40:40 v/v/v). A standard solution containing levels of fructose (2 µg/ml) and inositol (0.5 µg/ml) characteristic of the average seminal fluid and solutions containing half and twice these levels, were chromatographed in parallel with the unknown samples. The developed chromatograms were dried prior to staining with alkaline silver nitrate by the method of Trevelyan, Proctor & Harrison (1950) as modified by Lewin, Melmed & Bank (1974). The intensity of the silver stain on the Rf values of inositol and fructose were compared with the standards and were scored either +++ (high, greater than 3,000 µg/ml for fructose, or 700 µg/ml for inositol), ++ (average, approximately 2,000 µg/ml fructose or 500 µg/ml inositol), or + (lower than 1,000 µg/ml fructose or 250 µg/ml inositol).

PATIENT SELECTION

(a) Infertile Group:

Males were recruited voluntarily from the Southampton Infertility Clinic. The criteria for selection was as follows:

Firstly, a female factor had to be excluded and the following investigations were carried out.

Both multiparous and nulliparous female partners were accepted. Any female with a past history of chronic pelvic infection was excluded, as was anybody who had taken an oral contraceptive for up to two years prior to the start of investigation. A careful general examination was carried out to exclude any major medical disorders. A thorough vaginal examination was carried out, paying particular importance to the cervix, the size, mobility, position of the uterus and the normality of both adnexae. Any abnormality detected produced exclusion from the trial. Finally, a careful assessment of cervical mucus was carried out between days 12 - 14 of the menstrual cycle.

Further investigations included evidence of tubal patency either by dye insufflation at laparoscopy or hysterosalpingogram.

In the event of the latter, should any doubt of tubal patency have been found, the patient also underwent laparoscopy. Any signs of old pelvic infection in the form of adhesions excluded the patient from the investigation.

The following serum investigations were carried out:

(i) T3 and T4.

(ii) Serum prolactin - (levels up to 380 mIU/l were accepted.)

(iii) Plasma progesterone taken on day 21 by venepuncture on three consecutive cycles. All three progesterone samples had to show a level in excess of 30 nmol/l in order to show adequate luteal function.

Male selection was therefore carried out in couples where the female had completed the above criteria and where there had been a period of enforced infertility of at least two years' standing. No particular density count was selected, although a lower limit of 5 million/ml was established with the experience of the methodology of sperm velocity measurement. However, in view of the method of referral to the Southampton Male Infertility Clinic, on the basis of density counts of spermatozoa, there must be some element of bias in so far as sperm density is concerned.

Fifty such couples were screened for the above criteria and having satisfied the requirements, were entered into the

investigation. Further exclusions were also carried out at the time of the initial assessment, seeing the male partner in the first instance. Any signs of genital infection such as the presence of white cells or a cultured growth of bacteria in the ejaculate, further excluded these men from the investigation.

(b) Fertile Group (Controls):

Males were recruited for this group voluntarily, on the basis that their wives were pregnant at the time of sampling, the duration of which was not more than three months. The patients were recruited at the antenatal booking clinic and after full explanation, gave their informed consent for use in the investigation. Again, no particular density count was used in their selection. Twenty men were recruited to act as the control group.

After the initial visit, in order for the patients to acclimatise themselves to the environment and also to satisfy the above criteria, all seventy patients were asked to attend the University on two further occasions, within exactly one month of each other and asked to abstain from intercourse for exactly four days prior to the day of sampling. All specimens were produced in the same environment by masturbation into a sterile plastic container with a plastic lid. A timer was started at the time of ejaculation and the measurement for sperm velocity carried out exactly half-an-hour following ejaculation.

During this time the sample was kept at a constant temperature of 37°C in a water bath.

Prior to masturbation, venepuncture was carried out on all patients at each visit and 20 cc of blood was removed. This was allowed to clot at room temperature and the serum was removed. This was immediately frozen at -10°C.

Having completed the assessment of sperm velocity and carried out a standard seminal analysis which will be described in Chapter III, the seminal sample was centrifuged at 4000 revs per minute for 20 minutes. The seminal fluid was then removed and immediately deep-frozen to -20°C. The serum and seminal fluid samples were carefully labelled and were subsequently unfrozen for evaluation.

CHAPTER III

RESULTS

RESULTS

SPERM VELOCITY (μm/sec):

With reference to Table 3.1, the mean of the average velocity of the spermatozoa in the infertile group combining the first and second visit was 28.23 μm/sec with a standard deviation of 5.52 μm/sec. The mean of the average velocity of the spermatozoa in the fertile group - again combining the two visits - was 36.2 μm/sec with a standard deviation of 3.42 μm/sec.

There was a statistical significant difference between the fertile and infertile groups ($p = 0.0005$). A similar significant difference was found between the fertile and infertile groups at both visits (a) and (b) when considered separately.

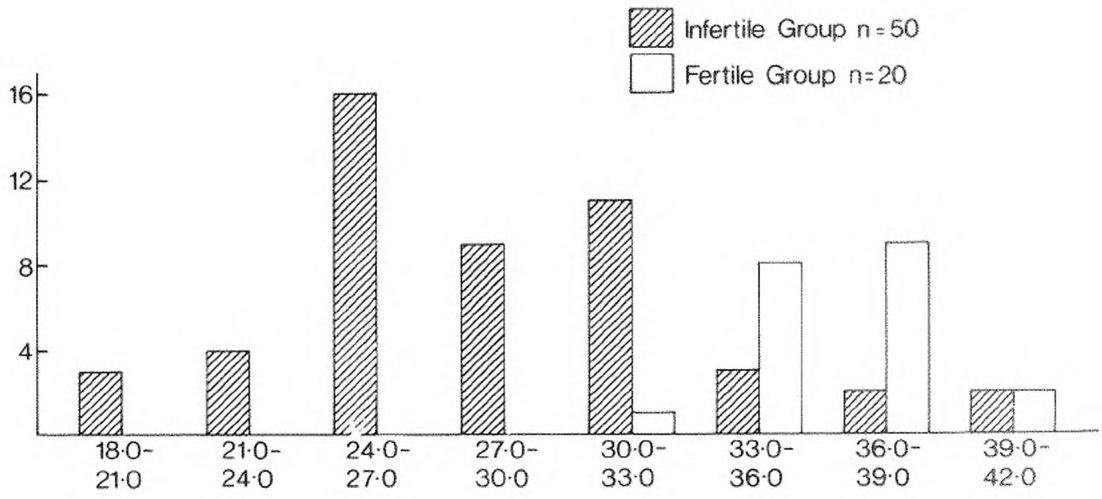
With reference to the histogram shown in Figure 3.1 showing the comparisons of mean velocities in μm/sec in the fertile and infertile groups, it is immediately apparent that all those of the fertile group are above 30 μm/sec. There are a few in the infertile group showing a velocity above 30 μm/sec, although a majority fall below this mark. Interestingly, during the next

Comparison of Mean Values (± SD) of Velocity (μm/sec) in Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	35.56	28.40	2.86	0.0005
± SD	3.07	5.19		
Mean of Visit B	36.89	28.06	2.53	0.0005
± SD	3.69	5.88		
Mean of All Samples	36.23	28.23	2.60	0.0005
± SD	3.42	5.52		

Table 3.1

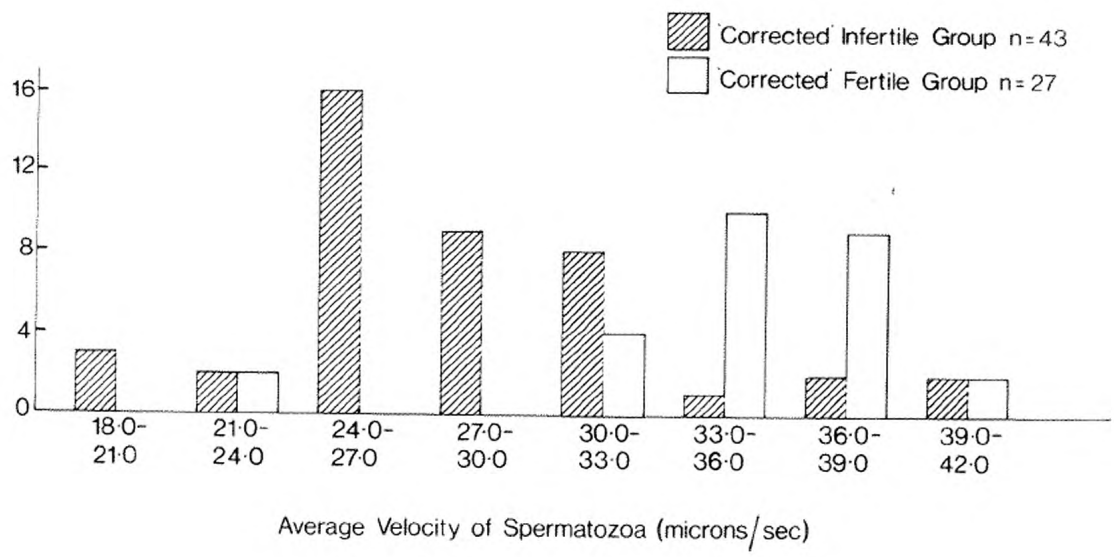
Figure 3.1 Comparison of Mean Velocities (microns/sec) in Fertile and Infertile Groups



six months when the patients were re-sampled at three-monthly intervals during a trial of medication (the basis of the second half of this Thesis) seven of the patients (14%) impregnated their wives. A "corrected" histogram is therefore shown out of interest in Figure 3.2. It must be remembered that half the group were taking medication and therefore no statistical analysis overall is appropriate. Also, it should be noted that some pregnancies occurred up to six months following initial sampling. Apart from two patients, the dividing line still remains at around 30 μm/sec and is certainly visually apparent from the histogram.

The mean velocity of these same patients, was 29.64 μm/sec which again confirms the apparent dividing line between fertile and infertile patients at around 30 μm/sec.

Figure 3.2 Comparison of Mean Velocities (microns/sec) in Fertile and Infertile Groups ('Corrected')



Comments:

From this data there is a definite significant difference between fertile and infertile patients with regards to sperm velocity which hitherto has not been established. That the spermatozoa of higher velocity should have a higher fertility potential is probably due to underlying biochemical efficiency brought about by correct maturation and initiation of motility.

Having therefore established the difference it is now appropriate to see how this parameter can be used in the assessment of the infertile male. Sperm velocity therefore will be compared with all the other parameters outlined in Patients and Methods and correlated individually.

Percentage Live/Dead:

This parameter is of dubious clinical significance although in rare cases total necrospermia can be found, but all such patients were excluded from the trial. It was thought worth including as a parameter in view of the fact that sperm velocity is a parameter only displayed by a live cell. The figures shown in Table3.2are the percentage number of dead cells in each group.

The mean percentage dead in the infertile group, considering all samples, was 16.7% with a standard deviation of 7.1%. This compares with a mean in the fertile group of 15.2% with a standard deviation of 6.3%. There was no statistical difference between the fertile and infertile groups when considering the mean of all samples or the mean of visit (a) and visit (b).

There was a significant correlation of a negative nature, between sperm velocity in μm/sec and % dead cells and this correlation persisted when considering the infertile group by itself ($p = 0.029$) but was absent in the fertile group ($p = 0.34$).

Comparison of Mean Values (± SD) of Percentage Dead Spermatozoa in Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	15.8%	17.0%	1.03	0.545
± SD	7.6%	7.7%		
Mean of Visit B	14.7%	16.3%	1.87	0.321
± SD	4.8%	6.6%		
Mean of All Samples.	15.2%	16.7%	1.28	0.271
± SD	6.3%	7.1%		

Correlation with Sperm Velocity (μm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	-0.17	0.02
Fertile (n = 20)	0.06	0.34
Infertile (n = 50)	10.18	0.029

Table 3.2

Comments:

Mean levels of 15 - 16% of dead spermatozoa are wholly to be expected from previous experience of Cockett (1975). He showed much higher values, though he measured the percentage viable, rather than the percentage dead. The mean level in his series was 52.2%, but interestingly, also showed that only in cases of extreme oligospermia (i.e. less than 10×10^6/ml) is there a significant rise in the levels of this parameter.

Percentage Motility:

This parameter at the present time is probably the most accepted indicator of potential fertility. It is however, a one-dimensional parameter, whereas velocity is two-dimensional. A significant difference between the two sub-groups (fertile and infertile) should therefore be expected. Whether these two parameters are correlated is still open to question. When considering the results it must be stressed that any patient showing agglutination of spermatozoa was excluded from the trial.

A second point to consider in comparing the results is that the estimation of motility in this case was made from photographs rather than from the standard subjective method in general practice made from a wet preparation (See Methods).

With reference to Table 3.3, the mean of the percentage motility in the infertile group was 61.4% with a standard deviation of 10.1%. The same levels were also found when considering the two visits separately - 60.9% and 61.9% respectively. In comparison, the mean percentage motility in the fertile group was found to be 62.7% with a standard deviation of 9.9%. The mean percentage motility in the first visit was slightly higher than 63.7%. As can be expected from these results, no statistical difference was found between the fertile and infertile groups ($p = 0.45$). There was however,

Comparison of Mean Values (± SD) of Percentage Motility In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	63.7%	60.9%	1.10	0.0293
± SD	10.2%	9.7%		
Mean of Visit B	61.8%	61.9%	1.13	0.959
± SD	9.8%	10.5%		
Mean of All Samples.	62.7%	61.4%	1.02	0.486
± SD	9.9%	10.1%		

Correlation with Sperm Velocity (μm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.39	0.0005
Fertile (n = 20)	0.22	0.078
Infertile (n = 50)	0.51	0.0001

Table 3.3

a statistical difference at the 5% level between the infertile and fertile groups at visit (a). This disappeared at visit (b) and did not persist as already stated when considering all the samples taken.

Turning to the correlation with sperm velocity, motility showed a positive correlation when all cases were considered ($p = 0.0005$). This correlation between motility and velocity was present in the infertile group ($p = 0.0001$) but not in the fertile group ($p = 0.07$).

Comments:

The overall mean percentage motility figures found in this study appear not to agree with those of Santomauro (1972), but do agree with Naghma-e-Rehan (1974) which showed a mean percentage of motility at 65%, although the standard deviation in his paper was up to 22%.

The fact that no significant correlation was found between motility and sperm velocity in the fertile group does suggest that these two parameters are of a separate nature. In contrast, the strong correlation found in the infertile group was thought to be due to problems of methodology in assessing motility in this manner. A certain density is required for accurate measurement and the presence of a number of low densities in the infertile group might be attributed to the apparent disagreement between the two groups. Further data will be presented later ( ) underlining the apparent difference between sperm motility and velocity.

Percentage Live Motility:

This parameter is a combination of percentage motility and percentage dead cells and gives an indication of the number of spermatozoa which are both alive and motile. With reference to Table 3.4, the mean percentage live motility in the infertile group was 73.8% with a standard deviation of 11.4% and for the fertile group it was 73.9% with a standard deviation of 9.8%. When considering the mean of both visits, there was no statistical difference in this parameter between the fertile and infertile groups and indeed the significance found at visit (a) with percentage motility disappears in this parameter when percentage dead cells is incorporated.

There was a strong correlation overall to sperm velocity ($p = 0.0003$) and also in both sub-groups, fertile and infertile ($p = 0.02$) and ($p = 0.0001$) respectively. However, again we must consider these results in relation to the reservations already considered in the previous Section concerning percentage motility.

Comparison of Mean Values (± SD) of Percentage Live Motility In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	75.6%	76.6%	1.27	0.477
± SD	9.9%	11.1%		
Mean of Visit B	72.3%	74.1%	1:47	0.532
± SD	9.71%	11.8%		
Mean of All Samples.	73.9%	73.8%	1.35	0.967
± SD	9.8%	11.4%		

Correlation with Sperm Velocity (μm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.33	0.00003
Fertile (n = 20)	0.30	0.026
Infertile (n = 50)	0.43	0.0001

Table 3.4

Comments:

The positive correlation between this parameter and sperm velocity would possibly be due to an exaggeration of the correlations already found with the two parameters percentage motility and percentage dead cells incorporated in this parameter. It is also interesting to note that like percentage motility, the correlation is much stronger in the infertile patients than the fertile patients. Again the problems with severe oligo spermia in this methodology for calculating percentage motility, are stressed.

One further point worth noticing is well displayed in the correlation of this parameter with sperm velocity. A high degree of statistical significance has been reported comparing all patients and the infertile patients, but the correlation does not appear to be quite so strong when considering the fertile patients. However, the r values in all three groups are very similar. The explanation for this apparent discrepancy is the variation in group size, thus the degrees of freedom. In terms of biological significance, the correlation found in the infertile group at the 5% level with an r value of 0.3 should be interpreted with some caution.

Sperm Density (million/ml) : Total Number of Spermatozoa Per Ejaculate.

Sperm Density:

The mean of sperm density in all samples in the infertile group was 39 x 10^6 with a standard deviation of 34 x 10^6. The mean density in the fertile group was however, 80 x 10^6 with a standard deviation of 42 x 10^6. There was a statistical difference between the two groups ($p = 0.0005$). This significant difference between the fertile and infertile groups persisted at both visits ($p = 0.0005$ and $p = 0.0005$). There was no statistical difference between the first and second visits in either the infertile or fertile groups when considered separately ($p = 0.07$ and $p = 0.58$). The distribution of sperm density in the fertile and infertile groups is shown in Figure 3.3.

When considering all patients, there was a strong correlation between sperm density and sperm velocity ($p = 0.0001$). Before interpreting this as an important connection, one must remember that there is a certain bias built in, in view of the manner of referral of patients and their subsequent selection. It has already been outlined in Chapter I that sperm density has a poor correlation to fertility. Many more patients, albeit with "normal density" yet apparently in an infertile situation, were not made available to the infertility clinic as they were not considered by their doctors to be partly to blame. Indeed, when studying the histogram shown in Figure 3.3 the expected high

Comparison of Mean Values ($\pm$ SD) of Sperm Density (x 10^6/ml) In Fertile and Infertile Patients.

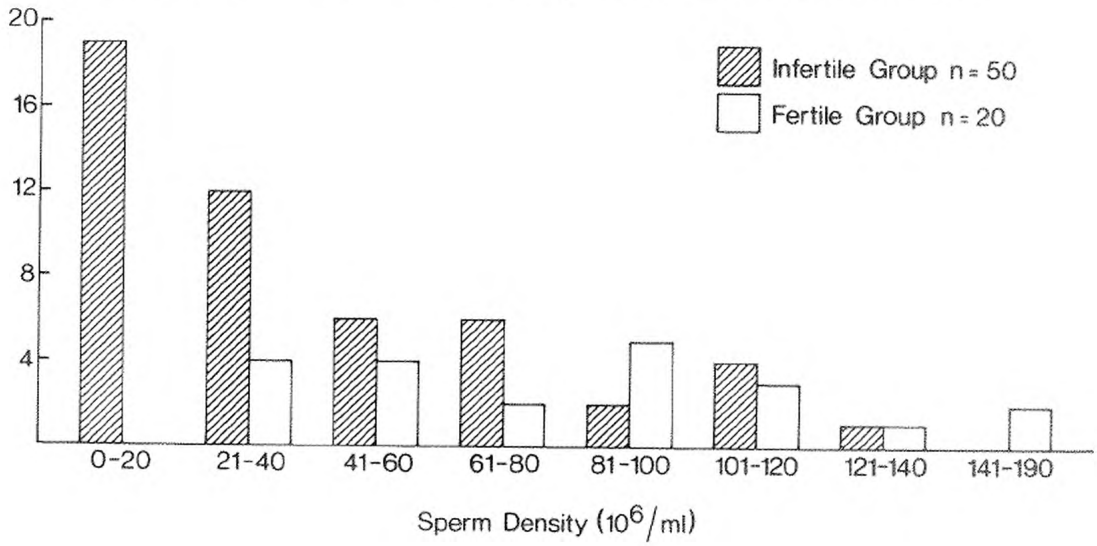
CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	$78x10^6$	$39x10^6$	1.62	0.0005
$\pm$ SD	$36x10^6$	$31x10^6$		
Mean of Visit B	$81x10^6$	$42x10^6$	1.56	0.0005
$\pm$ SD	$46x10^6$	$37x10^6$		
Mean of All Samples.	$80x10^6$	$39x10^6$	1.56	0.0005
$\pm$ SD	$42x10^6$	$34x10^6$		

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.376	0.00001
Fertile (n = 20)	0.055	0.365
Infertile (n = 50)	0.199	0.023

Table 3.5

Figure 3.3 Comparison of Sperm Density (10^6/ml) in Fertile and Infertile Groups



numbers of infertile patients with a low density, is quite apparent, though several lie above a density of 20 x 10^6/ml - the internationally accepted definition of oligospermia. In the fertile group there is a much wider range than previously expected - ranging from 30 x 10^6 - 190 x 10^6, which certainly underlines the fact that it is only at very low density levels that a significant difference appears between fertile and infertile patients.

Further evidence of this built-in bias is shown when considering correlation between sperm velocity and sperm density in the two separate sub-groups (infertile and fertile). In the infertile group there is a significant difference at the 5% level ($p = 0.023$) but in the fertile group there is no significant correlation shown ($p = 0.365$).

Comments:

The fact that the mean density expressed in million/ml in the fertile group was more than twice that of the infertile group (see Table 3.5) confirms the work of Macleod & Gold (1951 and 1956), Naghma-e-Rehan (1974) and Van Zyl (1972). The wide spread of results in the fertile group with a relatively large standard deviation tends to support the hypothesis that standard density is a poor parameter in the assessment of the infertile male. The mean sperm density of those patients whose wives did become pregnant in the infertile group was 32×10^6 with a standard deviation of 30×10^6 and there was no statistical difference between these patients and the remainder of the infertile group ($p = 0.36$).

This data supports the theory that a certain number of spermatozoa are required for fertilisation, although this number is very much lower than previously expected. It may be that the presence of a certain number of spermatozoa suggests that the seminiferous tubules are working properly and is only an indication of general testicular health rather than a specific parameter by which to judge fertility.

The lack of correlation between the sperm velocity and sperm density in the individual groups - fertile and infertile - tends further to stress the difference in these two parameters. It does have further significance, since

dilution of a sample prior to velocity estimation was carried out according to density (see Methodology) and if the strength of dilution is affecting velocity, the correlation would be expected to appear. This is encouraging since the lack of correlation in this parameter with sperm density confirms the experiments already described in the Methodology showing no effect on velocity with varying degrees of dilution.

Total Sperm Count (millions):

The mean of the total number of spermatozoa in the fertile group was 230 x 10^6 with a standard deviation of 158 x 10^6 while the mean of the total numbers in the infertile group was 127 x 10^6 with a standard deviation of 124 x 10^6. Similar to sperm density, there was a significant difference between the fertile and infertile groups at the 1% level, but again the interpretation of such data is dubious in view of the known bias in selection already discussed.

Furthermore, the mean total sperm count in the seven patients who impregnated their wives was found to be significantly different from the remaining infertile patients, but rather than being higher, the level was in fact lower.

The previous comments concerning sperm density and its correlation with sperm velocity are also true in this parameter since when considering all patients, there is a significant correlation ($p = 0.0005$). When the two groups are separated into fertile and infertile, the statistical difference disappears ($p = 0.22$ and 0.23 respectively) for the infertile and fertile groups.

Comparison of Mean Values ($\pm$ SD) of Total Numbers of Spermatozoa ($x10^6$) In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	$235x10^6$	$119x10^6$	2.22	0.004
$\pm$ SD	$153x10^6$	$102x10^6$		
Mean of Visit B	$225x10^6$	$135x10^6$	1'.37	0.028
$\pm$ SD	$168x10^6$	$143x10^6$		
Mean of All Samples.	$230x10^6$	$127x10^6$	1.63	0.0005
$\pm$ SD	$158x10^6$	$124x10^6$		

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.213	0.005
Fertile (n = 20)	-0.117	0.235
Infertile (n = 50)	0.07	0.22

Table 3.6

Comments:

Total sperm count is not a generally accepted parameter in assessing potential fertility in the male. The main use is assessing the total testicular output per ejaculate unaffected by the amount of seminal volume.

The apparent positive correlation with sperm velocity when considering all patients is not present when considering fertile and infertile separately. It must be stressed that this correlation only has an r value of 0.213 and in terms of biological significance should be interpeted with caution.

Total Number of Live Spermatozoa (millions):

The mean total number of live spermatozoa in the infertile group was 129 x 10^6 with a standard deviation of 108 x 10^6 and that for the fertile group was 193 x 10^6 with a standard deviation of 106 x 10^6. With an F value of 1.42 there was a statistical difference between the two groups ($p = 0.0005$). This statistical difference persisted at both visits (a) and (b) ($p = 0.0005$ and $p = 0.033$ respectively). As with the previous parameter (Total number of sperm) there was a statistical correlation between this parameter and sperm velocity ($p = 0.004$). This significant difference did not persist when considering the two sub-groups - fertile and infertile - ($p = 0.243$ and $p = 0.200$).

Comparison of Mean Values ($\pm$ SD) of Total Number of Live Spermatozoa ($x10^6$) In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	$195x10^6$	$98x10^6$	1.85	0.0005
$\pm$ SD	$119x10^6$	$88x10^6$		
Mean of Visit B	$190x10^6$	$115x10^6$	1'.26	0.033
$\pm$ SD	$141x10^6$	$126x10^6$		
Mean of All Samples.	$193x10^6$	$129x10^6$	1.42	0.0005
$\pm$ SD	$106x10^6$	$108x10^6$		

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.219	0.004
Fertile (n = 20)	-0.113	0.243
Infertile (n = 50)	0.084	0.200

Table 3.7

Comments:

The significant difference between the fertile and infertile groups is more due to the incorporation of sperm density than percentage dead in this parameter. The figures as far as correlation with sperm velocity is concerned, have the same reservations as those expressed with sperm density and sperm velocity. Since this parameter is very similar to the total number of motile spermatozoa, the significance of this parameter in the assessment of male infertility will be considered with the latter.

Total Number of Motile Spermatozoa (millions):

This parameter is a derivation of the total number of cells present, seminal volume and the percentage motility. With reference to Table 3.8 the mean of this parameter in the fertile group was 144 million with a standard deviation of 101 million and for the infertile group 75 million with a standard deviation of 65 million. There was a strong statistical difference between the two groups overall ($p = 0.006$) and also when considering the two visits separately ($p = 0.005$ and 0.008 respectively).

Similar to the previous parameter, there was a strong correlation between the total number of motile spermatozoa and sperm velocity when considering all patients ($p = 0.0001$). However, when considering the two sub-groups separately - infertile and fertile - there was a significant correlation at the 5% level in the infertile group, but no significance with the fertile group.

Comparison of Mean Values ($\pm$ SD) of Total Number of Motile Spermatozoa In Fertile and Infertile Patients. ($\times 10^6$)

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	152×10^6	71×10^6	3.13	0.0005
$\pm$ SD	105×10^6	59×10^6		
Mean of Visit B	136×10^6	78×10^6	1.94	0.008
$\pm$ SD	99×10^6	71×10^6		
Mean of All Samples.	144×10^6	75×10^6	2.39	0.0005
$\pm$ SD	101×10^6	65×10^6		

Correlation with Sperm Velocity (μm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.303	0.0001
Fertile (n = 20)	-0.089	0.291
Infertile (n = 50)	0.195	0.025

Table 3.8

Comments:

Since this parameter incorporates percentage motility, the same reservations and conclusions concerning the methodology used in calculating this parameter, especially in the infertile group, should again be stressed.

The lack of statistical correlation, especially in the fertile group, is consistent with previous data presented under the parameter of percentage motility. It would seem to indicate further the difference between sperm velocity and percentage motility.

Percentage Abnormal Forms:

This produced the least expected results since the mean of the abnormal forms in the fertile group (with reference to Table 3.9) was 43.2% with a standard deviation of 11.2%, while in the infertile group the mean was 39.1% with a standard deviation of 18.6%. This similarity was opposite to what was expected. The morphological estimations were all carried out by the same observer who undertakes the service commitment to the National Health Service in this area. As has already been discussed, it is a completely subjective parameter and varies from one laboratory to another.

There appears to be no statistical difference between fertile and infertile groups ($p = 0.115$) when considering all samples and this lack of significance persists at both visits (a) and (b) ($p = 0.356$ and $p = 0.179$ respectively).

Turning to the correlation with sperm velocity, the following data is found. In all cases there was a strong negative correlation ($p = 0.04$) between the two parameters. When separating the two groups the negative correlation persists in the infertile group ($p = 0.003$) but not in the fertile group ($p = 0.06$).

Comparison of Mean Values (± SD) of Percentage of Abnormal Forms In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	42.3%	38.5%	2.42	0.356
± SD	12.8	20.0%		
Mean of Visit B	44.1%	39.6%	3.23	0.179
± SD	9.6%	17.2%		
Mean of All Samples.	43.2%	39.1%	2.74	0.115
± SD	11.2%	18.6%		

Correlation with Sperm Velocity (μm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	-0.148	0.040
Fertile (n = 20)	-0.24	0.060
Infertile (n = 50)	-0.26	0.003

Table 3.9

Comments:

The overall high numbers in this particular series must reflect this particular observer's criteria and therefore must be interpreted with caution. The negative correlation found especially in the infertile group with reference to sperm velocity, tends to support the conclusion that morphologically normal spermatozoa have the highest velocity. However, in view of the low r value, it is probably better not to read too much biological significance into this correlation.

However, it was noticed that there was a progressive rise in the levels of abnormal forms as the investigation proceeded. The observer was not informed of this trend, but it would seem that the criteria for abnormality got progressively stricter.

Total Number of Normal Spermatozoa (millions):

The mean of the total number of normal spermatozoa in the infertile group was 85×10^6 with a standard deviation of 93×10^6 and the mean in the fertile group was 133×10^6 with a standard deviation of 99×10^6. There was a significant difference between the two groups ($p = 0.01$). The same reservations already apply to this parameter as to that of sperm density, since it is partially incorporated. The statistical difference persists at the first visit ($p = 0.014$) but disappears at the second visit ($p = 0.22$). In this particular group of samples the standard deviation is even larger - the infertile group being 104×10^6. Within the two groups - fertile and infertile - there was no statistical difference between the two samples taken at the first and second visit.

As far as correlation of the total number of spermatozoa with sperm velocity is concerned, a statistical significance was found when considering all patients ($p = 0.02$) but on separating the results into the two sub-groups the correlation disappears ($p = 0.19$ and 0.29 respectively). This underlines that the overall correlation is more a property of the total numbers involved than of the morphological nature of the spermatozoa present.

Comparison of Mean Values ($\pm$ SD) of Total Number of Normal Spermatozoa ($x10^6$) in Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	$142x10^6$	$82x10^6$	1.71	0.014
$\pm$ SD	$107x10^6$	$82x10^6$		
Mean of Visit B	$123x10^6$	$90x10^6$	1.30	0.22
$\pm$ SD	$91x10^6$	$104x10^6$		
Mean of All Samples.	$133x10^6$	$86x10^6$	1.12	0.010
$\pm$ SD	$99x10^6$	$93x10^6$		

Correlation with Sperm Velocity (μm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.165	0.025
Fertile (n = 20)	-0.087	0.296
Infertile (n = 50)	0.085	0.199

Table 3.10

Comments:

Few definite conclusions can be extracted from the above data, especially in view of the lack of previous work with this particular parameter. Again, as with percentage abnormal forms, the above data does support the conclusion that morphologically normal spermatozoa have a higher velocity. The high values for abnormal forms shown in this series tend to support the work of Freund (1966). In spite of the work of Eliasson (1971) who produced some form of criteria for the assessment of the abnormal spermatozoon, the laboratory involved in this particular series used a wet preparation which might explain these particularly high results. The fact that the results at both first and second visits were very similar suggests that an adequate space of time was selected between the samples in order to avoid the ageing process known to occur. The mean of the percentage of abnormal forms and the total number of abnormal forms of those patients in the infertile group who impregnated their wives, was not significantly different from that of the overall infertile group, which again underlines the precarious nature of using percentage abnormal forms or total number of normal spermatozoa as a fertility parameter.

Seminal Volume:

The mean of the seminal volume in the infertile group was 3.3 ml with a standard deviation of 1.3 ml. The mean of the seminal volume in the fertile group was 2.9 ml with a standard deviation of 1.2 ml. There was no significant difference between the two groups when considering the mean of the two visits or considering the mean of all samples. There was also no significant difference between the two groups when separating the results into visits (a) and (b), ($p = 0.39$ and 0.17 respectively).

The results as far as correlation with sperm velocity is concerned are more interesting with an overall negative correlation ($p = 0.01$). When separating the two groups into infertile and fertile, this negative correlation was not found in either group at the 5% level.

Comparison of Mean Values (± SD) of Seminal Volume

In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	3.08	3.37	1.25	0.395
± SD	1.19	1.33		
Mean of Visit B	2.8	3.3	1.01	0.177
± SD	1.3	1.3		
Mean of All Samples.	2.9	3.3		
± SD	1.2	1.3	1.11	0.155

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	-0.183	0.018
Fertile (n = 20)	-0.240	0.067
Infertile (n = 50)	-0.107	0.144

Table 3.11

Comments:

It appears therefore that infertile specimens have a larger ejaculatory volume and are associated with a lower average velocity of spermatozoa in the ejaculate. This slightly larger seminal volume agrees with the work of Macleod & Gold (1951). This apparent difference between the two groups brings up the possibility of either over-production of those glands contributing to the ejaculatory volume or some alteration in the chemical constituents of the seminal fluid which could affect sperm velocity.

Serum Hormonal Estimations.

Follicle Stimulating Hormone (FSH) (IU/1):

The mean serum FSH in the infertile group (with reference to Table 3.12) was 3.4 IU/1 with a standard deviation of 2.6 IU/1. The mean of the fertile group was 2.8 IU/1 with a standard deviation of 2.2 IU/1. There was no statistical difference between the two groups either when considering the means of the two samples or the two samples separately ($p = 0.27$ and 0.154 respectively). When separating the two visits, one interesting fact did appear. At the second visit (b) the mean FSH for the infertile group was 4.2 IU/1 with a standard deviation of 2.9 IU/1 and that of the fertile group was 2.6 IU/1 with a standard deviation of 2.1 IU/1. In this case there was a statistical difference between these two groups ($p = 0.038$).

Also when looking at the infertile group by itself, there was a statistical difference in the FSH levels between the two visits (a) and (b) ($p = 0.0005$), the value for the second visit (b) being much higher. This difference between the two visits was not noticed in the fertile group. Taking into account the inter-assay coefficient of variation, the difference persists.

Comparison of Mean Values (± SD) of Serum FSH (IU/1)

In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	2.9	2.7	1.12	0.768
± SD	2.2	2.1		
Mean of Visit B	2.6	4.2	1.88	0.038
± SD	2.1	2.9		
Mean of All Samples.	2.8	3.4		
± SD	2.2	2.6	1.48	0.154

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.036	0.336
Fertile (n = 20)	0.118	0.234
Infertile (n = 50)	0.137	0.086

Table 3.12

FSH was not positively correlated to sperm velocity in either the overall group or in the two sub-groups - infertile and fertile. The sperm density situation was slightly different, as there was a negative correlation in both fertile and infertile groups against FSH ($p = 0.04$ and 0.05 respectively). This finding is in agreement with de Kretser (1972) and Franchimont (1972) but not with Fossati (1979) who found no correlation.

Turning to sperm motility, FSH was again correlated to this parameter but only in the fertile group ($p = .03$). Other interesting correlations were found between FSH and LH in the infertile group ($p = 0.0001$) although not in the fertile group ($p = 0.07$).

Comments:

That FSH correlates with sperm density, particularly in the fertile group in a negative fashion, is wholly to be expected, although from the work of Jackaman (1977) a closer correlation would be expected between FSH and density in the infertile group. In this series there was a negative correlation but it was not statistically significant ($p = 0.07$). None of the patients of the infertile group had a level of more than twice normal, which certainly from the work of de Kretser (1972) excludes any patients in this series having serious testicular disorder. There was also no evidence of spermatogenic arrest in any of our fifty infertile patients before the stage of spermatid formation. It is because of this that the high levels found by Franchimont (1972) and Hunter (1974) are not apparent in this series. The fact that the mean level FSH in the infertile group was slightly higher than the fertile group is to be expected. In contrast, no patient had a serum FSH level of less than 0.8 IU/l which would have suggested some disorder of pituitary function (Lunenfeld, 1974).

These levels are in agreement with Fossati (1979) although his groups were based on density levels rather than fertility status. His level of 1.8 IU/l for the normospermic group was statistically different from the oligospermic group with values of 3.8 IU/l. Franchimont (1972) and Hopkinson (1977) also showed similar levels.

The difference in the correlation of sperm motility and sperm velocity to FSH is interesting. Since FSH is known to be important in the whole process of spermatogenesis and that motility is related to a certain maturity, this correlation is to be expected. However, the fact that sperm velocity does not correlate with FSH shows that although FSH is required to produce the structure necessary for motility, it plays no part in the efficiency of that motility and hence the sperm velocity, This is just another indication of the difference between sperm velocity and sperm motility.

Luteinizing Hormone (LH) (IU/1):

The mean serum LH in the infertile group was 5 IU/1 with a standard deviation of 1.9 IU/1 and the mean of the fertile group was 5.2 IU/1 with a standard deviation of 1.8 IU/1. There was no statistical difference between the groups, either when considering the mean of all samples ($p = 0.58$) or the mean of each individual visit (a) and (b) ($p = 0.33$ and 0.86 respectively).

No statistical correlation with sperm velocity was found in either the group containing all patients or in the infertile group or fertile group. LH also had no statistical correlation with sperm motility in either the fertile or infertile groups ($p = 0.07$ and 0.47) showing that unlike FSH, LH does not appear to play a direct role in the maturation and hence motility of the spermatozoa.

There was a strong negative correlation between serum LH and density in both groups, the strongest being in the fertile group ($p = 0.0001$). As already stated LH correlated with FSH in the infertile group strongly ($p = 0.00001$) but not in the fertile group ($p = 0.7$). There was no correlation between LH and dihydrotestosterone, but in contrast LH did show a correlation with oestradiol in a negative fashion, but only in the fertile group ($p = 0.05$). The r value was low and therefore probably not of biological significance.

Comparison of Mean Values (± SD) of Serum LH (IU/l)

In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	5.2	4.7	1.47	0.339
± SD	1.6	1.9		
Mean of Visit B	5.1	5.2	1.26	0.861
± SD	2.0	1.8		
Mean of All Samples.	5.2	5.0	1.10	0.581
± SD	1.8	1.9		

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	-0.067	0.212
Fertile (n = 20)	-0.063	0.347
Infertile (n = 50)	-0.132	0.093

Table 3.13

Comments:

The results for serum luteinizing hormone in this series were higher than those found by Fossati (1979) although his group allocation was different. The correlation between serum LH and sperm density does not agree with Franchimont (1972), Hopkinson (1977), Millett (1972) or Rosen (1971). There is agreement, however, with Hunter (1974) who did show a correlation between LH and sperm density. The correlation between FSH and LH, especially in the infertile group appears to agree with Franchimont (1972) and de Kretser (1972). The latter suggested that their findings showed a synergistic effect of FSH and LH and that there was a gradual increase in their levels with severe testicular lesions. Since all patients with severe testicular disorders were excluded from this series, no comment as to the above statement can be made. Certainly, in this series the overall impression of previous work - that disorders of testicular function are associated with mildly elevated levels of LH, is not borne out.

The lack of correlation with dihydrotestosterone was unexpected, especially in view of the work of Jackaman (1977) comparing low levels of serum testosterone with elevated levels of serum LH in 30% of the males studied. It must be remembered that serum testosterone in the seminiferous tubules can be reduced to either dihydrotestosterone or aromatized directly to

oestradiol. This might explain the apparent lack of correlation to LH. Certainly the correlation between oestradiol and LH in the fertile group ($p = 0.05$) suggests that there may be differences in the metabolism of testosterone between fertile and infertile patients.

The lack of correlation of LH to sperm velocity is unremarkable. Many patients are known to have normal androgen status and yet present with reduced sperm motility.

Dihydrotestosterone (DHT) (nmol/l):

Dihydrotestosterone was chosen as the main androgen marker rather than testosterone, since it had already been established that the latter had poor correlation with fertility (see Chapter I). Dihydrotestosterone is a direct metabolite of testosterone from the process of reduction occurring both in the seminiferous tubules and in the epididymis. However, as stated in the Review of Literature, the origin of plasma dihydrotestosterone is somewhat different, the majority being produced in tissues outside the testis and accessory glands.

With reference to Table 3.14 the mean serum DHT in the infertile group was 2.78 nmol/l with a standard deviation of 1.14 nmol/l and that for the fertile was 2.98 nmol/l with a standard deviation of 0.8 nmol/l. There was no statistical difference between the fertile and infertile groups when considering the mean of all samples ($p = 0.269$) or at the mean levels at each individual visit (a) and (b) ($p = 0.54$ and 0.543 respectively). There was also no statistical difference within each group between visits (a) and (b).

There was no statistical correlation between DHT and sperm velocity in either infertile or fertile groups or in all patients considered. There was a negative correlation between DHT and sperm density in the infertile groupe at the 5% level but not in the fertile group. There was also a strong correlation between

Comparison of Mean Values (± SD) of Serum DHT (nmol/1)

In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	3.0	2.8	1.90	0.542
± SD	0.9	1.2		
Mean of Visit B	2.9	2.7	1.71	0.436
± SD	0.7	1.0		
Mean of All Samples.	2.98	2.78	1.85	0.269
± SD	0.8	1.14		

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	-0.011	0.445
Fertile (n = 20)	0.177	0.136
Infertile (n = 50)	-0.120	0.116

Table 3.14

motility in the fertile group ($p = 0.0001$) which appears to confirm the hypothesis that this parameter plays an active role in the epididymis. There was no correlation between dihydrotestosterone and FSH in either group or with LH. There was a statistical correlation between DHT and oestradiol ($p = 0.001$) but only in the infertile group.

The mean level of DHT in the seven patients who impregnated their wives was 2.00nmol/lwhich appears to be opposite the trend in the fertile group. The difference between this group and the infertile group was statistically significant ($p = 0.001$).

Comments:

The lack of significant difference between infertile and fertile patients tends to suggest that this parameter is of little importance in the assessment of the infertile male. However, as has already been stated in the Review of the Literature, production of dihydrotestosterone is different at the plasma level as compared to the seminal fluid level. At the same time it must also be remembered that dihydrotestosterone only crosses the blood-testis barrier in very small amounts (Setchell & Main, 1975). The fact therefore that higher levels of serum DHT appear to occur in lower sperm densities is probably a quirk of statistical analysis rather than of biological significance. Possibly the lower levels found in the seven successful patients is also explained in this manner. As far as this parameter is concerned, i.e. DHT, more emphasis should be laid on its action in the seminal fluid. Overall, the results tend to agree with De Aloysio (1978) who also showed no appreciable changes in plasma dihydrotestosterone in normo and oligospermic patients.

Serum Prolactin (mIU/1):

Due to the interest in this parameter in the field of female infertility, hypotheses have been put forward that a similar role may be found in the male. The second half of this Thesis is involved in the assessment of serum prolactin in spermatogenesis. The following will be the overall results in relation to sperm velocity.

The mean serum prolactin of the infertile group was 220.1 mIU/1 with a standard deviation of 84.8 mIU/1 and that of the fertile group was 219.9 mIU/1 with a standard deviation of 97.5 mIU/1. As can be seen from Table 3.15 there is no statistical difference between the two groups ($p = 0.98$). This is also true when looking at the two visits separately (a) and (b) ($p = 0.98$ and 0.95 respectively). Looking at each group separately, there is no difference between the visits (a) and (b).

There was no statistical correlation of serum prolactin to sperm velocity either when considering all patients ($p = 0.11$) or in the fertile ($p = 0.30$) or infertile groups ($p = 0.07$). Prolactin also showed no positive correlation to either sperm density or sperm motility in either of the two groups, although a positive correlation was found between FSH and serum prolactin in the infertile group ($p = 0.01$). The significance of this will be discussed later in the second half of the Thesis. There was also

Comparison of Mean Values (± SD) of Serum Prolactin (mIU/1) In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	228.3	227.7	1.26	0.982
± SD	103.7	92.4		
Mean of Visit B	211.5	212.6	1.47	0.958
± SD	92.8	76.6		
Mean of All Samples.	219.9	220.1	1.32	0.986
± SD	97.5	84.8		

Correlation with Sperm Velocity (µm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	0.101	0.116
Fertile (n = 20)	0.081	0.309
Infertile (n = 50)	0.144	0.076

Table 3.15

no statistical correlation found between serum prolactin and serum LH, oestradiol and dihydrotestosterone, in either the fertile or infertile group.

Comments:

As far as this series is concerned, there appears to be no difference in the serum prolactin levels with relation to fertility. The lack of any statistical difference between the two visits (a) and (b) also removes the possibility of any stress effects. Further comments on the above results in correlation to other paramters will be discussed in the second half of this Thesis.

Serum 17β Oestradiol (pmol/l):

The mean serum 17β oestradiol (with reference to Table 3.16) in the infertile group was 168.85 pmol/l with a standard deviation of 68.5 pmol/l and that of the fertile group was 139.37 pmol/l with a standard deviation of 68.8 pmol/l. There was a statistical difference between the infertile and fertile groups ($p = 0.023$) a fact not established in any previous report. Looking at the two visits separately, there was no significant difference between the fertile and infertile groups at visit (a) ($p = 0.41$) but there was at the second visit (b) ($p = 0.015$). Taking the two groups separately and looking at the difference between visit (a) and (b) there is a significant difference in the fertile group ($p = 0.012$) but not in the infertile group ($p = 0.11$).

In respect of correlation with sperm velocity there was no correlation in the group considering all patients ($p = 0.249$) or in the two main sub-groups - fertile ($p = 0.45$) or infertile ($p = 0.18$). In other parameters serum 17β oestradiol showed no correlation to serum LH, FSH or prolactin, but there was an interesting correlation with serum dihydrotestosterone in the infertile group ($p = 0.001$) although no such difference was found in the fertile group ($p = 0.19$). In contrast, serum 17β oestradiol showed correlation with LH in the fertile group ($p = 0.05$) but not in the infertile.

Comparison of Mean Values (± SD) of Serum 17β Oestradiol (pmol/1) In Fertile and Infertile Patients.

CLASSIFICATION	Fertile (n = 20)	Infertile (n = 50)	F Value	Significance (p =)
Mean of Visit A	161.25	176.30	1.22	0.416
± SD	64.41	71.28		
Mean of Visit B	117.50	161.4	1.06	0.015
± SD	67.63	65.57		
Mean of All Samples.	139.37	168.85	1.01	0.023
± SD	68.8	68.5		

Correlation with Sperm Velocity (μm/sec).

CLASSIFICATION	r Value	Significance (p =)
All Patients (n = 70)	-0.057	0.249
Fertile (n = 20)	-0.016	0.459
Infertile (n = 50)	0.091	0.181

Table 3.16

There was also a statistically significant correlation in the infertile group between serum oestradiol and sperm density ($p = 0.05$). This information certainly suggests that oestradiol plays an important part in the role of spermatogenesis. Finally, no correlation was found between oestradiol and sperm motility, which therefore suggests that this parameter has little effect on the initiation of motility in the epididymis.

When considering the seven patients in the infertile group who impregnated their wives, there was a lower mean oestradiol level (150 pmol/l) but not significantly different from the remaining infertile ($p = 0.26$) or fertile groups ($p = 0.6$).

Comments:

As already discussed in Chapter I, oestradiol is known to be produced by the testis by the presence of specific receptors for this steroid in the Leydig cells (Mulder, 1976). Franchimont et al (1975) suggested that oestradiol may have an important effect on the secretion of FSH. In rats oestrogen is known to have the potential for directly affecting the pituitary response to LRH (Labrie, 1978). This might explain the apparent connection between oestradiol and LH in the fertile group, though the correlation was somewhat weak. If the higher levels of oestradiol found in the infertile patients in the serum are interfering in some way with the testicular feed-back to the hypothalamus, one would expect lower levels of the gonadotrophins, FSH and LH to be present. As this was not so, the biological explanation of the difference in oestradiol plasma levels between fertile and infertile, remains unexplained. One can therefore only point out an interesting difference and agree with Tresguerres (1981) that the role of oestradiol in the aetiology of male infertility needs further investigation.

CHAPTER IV

DISCUSSION

Time-lapse Photography and its Measurement of Sperm Velocity:

Time-lapse photography, rather than being a completely new form of methodology, is a further development of various aspects of previous methods used to measure sperm velocity. It certainly is quite different to the method used by Harvey (1960) though the actual results obtained are somewhat similar. The basis of the method comes from the original work by Rothschild (1951) later modified by Rikmenspoel (1956). The work of Janick (1970) also produced a methodology very similar, in that the time-delay principle was used, although only of one second, and a similar speed of film was used. He used a diluent and a microscope adapted for dark-field illumination but did not use negative-phase light. All other methods using time-delay photography including, to a certain extent, the method of Makler (1979a & b) with a multiple-exposure technique, concentrated on the movement of the head. The methodology described here was solely concerned with the movement of the tail. It is for this reason that clearer pictures were obtained than from our predecessors in this field.

Effect of Dilution:

In time-lapse photography there appears to be no interference in the measurement of sperm velocity according to the strength of a certain dilution. However, diluents with markedly contrasting viscosity do significantly alter the velocity and therefore levels obtained of sperm velocity are only comparable if the same type of diluent is used in each case. This cannot be stressed too strongly. In Janick's methodology in 1970 he also showed that increasing the dilution with citrate egg-yolk diluent had no appreciable effect on sperm velocity. Makler in 1979c claimed that by diluting, one introduced several variables:

"1) The viscosity of the medium is generally lowered by the diluent and therefore the resistance to the swimming spermatozoa is reduced;

2) the concentrations of metabolites and nutritive and other organic and inorganic substances are altered so that motility may be stimulated or depressed;

3) distances between spermatozoa are increased, thus enabling the sperm to swim freely and progressively without colliding or jostling with each other".

In this paper, using the methodology of multiple-exposure photography he showed the effect of sperm velocity before and after

dilution of the semen with seminal plasma or normal saline in a ratio of 1:3. It does appear from his work that the addition of seminal plasma or normal saline, has produced higher values of sperm velocity. It is interesting to note from his work that these dilutions only had an effect on sperm velocity and not sperm motility, again emphasizing the difference in these two parameters.

In this series, all samples were diluted at varying concentrations. It must be stressed here that there are bound to be different levels of sperm velocity expressed according to both the different methods used and the different laboratories undertaking the work. The most important thing is the comparison of the results between fertile and infertile patients rather than the actual individual values themselves.

Makler (1979c) did accept in his paper that seminal fluid is not an ideal environment for the moving spermatozoon and added that it is: - "unjustified to determine spermatozoa motility only in the seminal fluid".

Janick in his paper in 1970 also put this point, pointing out that in view of the fact that spermatozoa are found in the cervical mucus within 90 seconds following intercourse, any measurements of motility or any other function of human spermatozoa in seminal plasma beyond a few minutes after ejaculation

should not be considered as physiological. He ended by saying:- "but in assessing male fertility potential, we have little choice, because the use of any other fluid medium would be equally unphysiological". At that time he did not have the work of Lopata (1976) who was able to extract human tubal fluid and to re-constitute a diluent exactly similar to it.

In conclusion, one can say that the combination of time-lapse photography depending on movement of the tail more than the head, coupled with the use of a sperm diluent similar to human tubal fluid appears to have no acceleratory or deceleratory effect on sperm velocity, but will give comparatively reliable results.

Repeatability of Time-Lapse Photography:

As shown in Experiments 5 and 6, the coefficient of variation for this methodology is extremely low at 3.2 - 4.6%. This low coefficient of variation makes comparison of the results achieved by this method more pertinent. In other methods reporting the measurement of sperm velocity, no such coefficient of variation is mentioned and thus comment is not possible. It certainly is very much better than that found in the objective methods of studying sperm motility.

The Effect of Temperature on Sperm Velocity:

The progressive increase in sperm velocity from 25°C to 40°C is confirmed by the work of Janick (1970) who measured sperm velocity at both room temperature and 37°C. However, his levels at both of these temperatures are slightly higher than those recorded with time-lapse photography. As already stated, comparisons between the velocities of two different methodologies is not rewarding. However the similar trend found in both sets of figures must confirm the effect that temperature has on the biochemical process that brings about sperm movement and sperm velocity.

The Effect of pH on Sperm Velocity:

The results shown in Experiment 8 were consistent with the hypothesis that sperm activity is brought about by an enzymic process. Only one other paper mentions the effect of pH on sperm velocity and that is the work of Harvey (1960) who showed very similar results to ours with definite fall-off in sperm velocity with progressive acidity. Standardization of pH in all measurements is essential in order that the results can be comparable.

Conclusions on the Methodology of Time-lapse Photography:

With the addition of the works of Katz & Dott (1975) which provided a method for reasonably quick and accurate measurement of the photographic fields achieved, finally makes time-lapse photography an effective and accurate method of assessing sperm velocity. The method is economically viable, with the results obtained reasonably quickly. Using the sperm diluent as described in all cases, solves the problem of seminal debris and also the wide range of sperm densities present in clinical practice. In the lower density ranges - i.e. less than 5 million/ml fears that the accuracy of the measurements might not be quite so good have been put forward by Makler (1979 a)in his method and also by Janick (1970). Obviously at this level of sperm density, some form of severe disorder of spermatogenesis is taking place and other methods of assessment such as serum FSH and testicular biopsy have a more important prognostic value than sperm velocity.

Results of Time-lapse Photography as Compared to Other Methodologies:

Table 4.1 : Results from Various Studies on Velocity of Human Spermatozoa.

Author, year.	Method	Spermatozoal velocity	
		Average	Upper limit
		μm/sec	
Harvey (1960)	Sperm dilution, Stopwatch, counting chamber.	45	90 - 100
Janick and Macleod (1970)	Sperm dilution, 1-sec exposure, photography.	16 - 36	55
Bartak (1971)	Undiluted sperm, counting chamber, stopwatch, nomogram.	30.8[a]	100[a]
Sokoloski et al (1977)	Turbidimetric, diluted sperm	100	280
Makler et al (1979a)	Undiluted sperm, multiple exposure, photography.	30.3	80 - 90

[a] Figures indicate average speed of all spermatozoa (motile and non-motile); average speed of motile spermatozoa is about 50% higher.

As can be seen from the above Table, there appears to be quite a wide variation in the levels obtained by different methods. Two main points must be kept in mind when studying this Table; firstly,

some methods measure the speed of all spermatozoa while others only the average speed of the motile spermatozoa, as is the case in our methodology. Secondly, as stated previously, comparisons between methodologies are not as rewarding as comparisons within one methodology between two different clinical sub-groups - i.e. fertile and infertile.

Sperm Velocity as Measured by Time-lapse Photography in Comparison to Other Parameters Considered:

In this Thesis the important correlations found between sperm velocity and other parameters were as follows:

(a) Percentage Dead Cells:

This negative correlation is wholly to be expected, since only the live cells can show the parameter of sperm velocity.

(b) Percentage Motility:

The contrasting results of the fertile and infertile groups, the correlation of sperm velocity to percentage motility is further confused by looking at other reports. Freund (1962) did show a correlation ($r = 0.76$) as did Naghmar-e-Rehan (1975) ($r = 0.70$). However, the later work of Makler (1979c)claims that the percentage of motile spermatozoa and their velocity are two specific independent parameters and certainly he shows this from his work. He did not find a correlation between the two parameters at the 5% level and at the same time criticised Bartak's sperm velocity test which was a combination of spermatozoal speed and percentage motility. Macleod (1969) also used a percentage of motile spermatozoa and their motility grade (+ to ++++) as the motility index and suggested 120 or above as a good index. However, Makler (1979b)correctly criticised this since these were arbitrary numbers without any mathematical meaning and only represented relative values. Taking into account the fears already expressed

concerning the accuracy of this measurement in low sperm densities, it would tend to tilt the evidence away from the infertile group and suggests that the fertile group is probably more correct.

Sperm Density:

The lack of correlation between this parameter and sperm velocity as measured by TLP, underlines the fact that the latter is a separate parameter. Harvey (1959) unfortunately made no mention of a correlation of these two parameters. Naghmar-e-Rehan (1975) using a progressive motility parameter showed a weak correlation with sperm density, although the work of Freund (1962) using a similar parameter, did suggest a slightly stronger connection ($r = 0.42$). The sperm velocity test reported by Bartak (1971) suggests a very much stronger correlation between sperm velocity and sperm density. As already stated, his patients were divided into normospermic and oligospermic, the dividing line at 40 million/ml. The mean sperm velocity level at one hour in the normospermic group was 30.8 um/sec, while the oligospermic group at one hour was 4.4 um/sec. The difference between these figures was statistically significant.

Janick & Macleod (1970) using a photographic method, unfortunately made no reference in their results of sperm velocity as compared to sperm density.

Makler (1975a), using a multiple exposure technique, only showed a weak correlation when measuring sperm velocity and correlating it to sperm density ($r = 0.30$).

Therefore it appears from the above experience and present data that sperm velocity appears not to correlate with sperm density, certainly not in a manner which would be of biological significance.

Percentage Abnormal Forms:

The evidence from this series in as far as the negative correlation between percentage abnormal forms and sperm velocity indicates that morphologically normal spermatozoa have a higher velocity. This has previously been suggested by Bartak in 1971 who showed that when separating his oligospermic group - (i.e. less than 40 million/ml) into those showing good morphological features and those showing bad morphological features, there is a significantly higher velocity in the group showing better morphology. Janick (1970) (his method depends upon photography of the spermatozoal head) noted that at very low sperm densities, a larger number of abnormal head forms were seen and commented that this might be the reason for his less reliable results at that end of the density range.

In assessing the percentage of abnormal forms it is difficult to divide the immature but morphologically normal spermatozoa from the abnormal but mature spermatozoa, since the ability to move will not appear until a certain stage of maturity. Obviously this is a pertinent point, as the evidence in this series and other work previously, certainly suggests that the morphologically normal spermatozoa have a higher velocity. Indeed the correlation found between sperm velocity and the total number of normal cells in the ejaculate when considering all the patients together, would further support the above hypothesis.

Seminal Volume:

The trend for higher seminal volumes in the infertile patients might suggest some disorder in the various glands which contribute not only to the volume but also to the chemical constituents of the seminal volume. The correlation noticed between seminal volume and sperm velocity when considering all patients ($p = 0.018$) lends further support to the hypothesis that sperm velocity can be influenced by the state of health of the various glands - e.g. seminal vesicles and prostate. However, it must be noticed that this correlation was only found at the two per cent level and therefore should be interpreted, in terms of biological significance, with caution.

Sperm Velocity and Serum Hormonal Measurements:

With reference to Chapter III, sperm velocity was shown not to correlate with any of the following serum measurements: FSH, LH, DHT, prolactin and oestradiol. Certainly in respect of the first two - FSH and LH this lack of correlation is not too surprising, since both are more involved in one way or the other in the maturation of spermatozoa. Motility and hence velocity only occurs at the time of ejaculation, the process of which has already been summarised in Chapter I . In a severe case of hypogonadotrophic hypogonadism, with subsequent lack of proper development of the accessory glands, the effect on sperm motility may be expected. Since none of these patients were incorporated in this Thesis it is not surprising therefore that no correlation exists.

Lack of correlation with serum oestradiol in either of the two sub-groups is disappointing especially in view of the significant difference found between fertile and infertile patients both in the parameter of oestradiol and the parameter of sperm density. Serum prolactin came closest to being correlated with sperm velocity in the infertile group. (Prolactin will be discussed more fully in Part II of this Thesis).

In view of the fact that serum DHT is only a reflection of peripheral metabolism, the lack of correlation between this parameter and sperm velocity is not surprising. The significance

of the above results is further complicated by the fact that no previous workers who have looked at sperm velocity as a parameter in male infertility, produced any evidence including serum hormone and measurement.

It seems therefore that sperm velocity is unaffected by the serum levels of these hormones described. This however might be explained by the possibility that serum levels are not accurately reflecting the conditions at testicular level.

Sperm Velocity (TLP) and the Relationship to Fertility:

The addition of a further parameter in the assessment of the infertile male is only worthwhile if that parameter can be shown to have some form of correlation to fertility. The strong statistical difference between the velocities in fertile patients compared to infertile patients definitely suggests that this parameter could become a useful clinical tool. This information is further strengthened on recalling the strict criteria laid down for the definition of a fertile patient and more so by not using sperm density as a selection criterion in the infertile patients. It must be stressed again that none of the wives of the patients after full investigation showed any barrier to fertility. Further to their initial investigations, they were further monitored over a period of six months, including serum progesterone carried out on the twenty-first day of alternate months over a six-month period.

The histogram shown in Figure 3.1 comparing the mean velocities in μm/sec between the fertile and infertile groups shows a definite cut-off point at 30μm/sec. Unfortunately, no other methods used in assessing sperm velocity have tested their results in terms of fertility. This should be taken into account when comparing these results with those of other workers. The closest paper to correlating to some form of fertility in fact was Harvey's in 1959. Using her own fertility index (Harvey 1953) she showed higher sperm velocities in those patients with an index of 50 or over. Those patients showing less than 30 in her

fertility index showed a distribution much lower down the velocity range and interestingly, the majority of these patients were below 30μm/sec.

She did include nine samples from men of proven fertility (although omitting any definition of fertility). A majority of these gentlemen had sperm velocities in excess of 30 μm/sec. She concluded that the speed of spermatozoa may be a factor in differentiating between the fertility of semen samples which on the basis of density, motility and sperm morphology, appear very similar.

Bartak in 1971 expressed his results in three groups - normospermic, asthenospermic and oligospermic. He showed a significant difference between asthenospermics and oligospermics when compared to normospermics and a far greater fall-off in sperm velocity in these groups when measured at three and five hours following ejaculation. Interestingly, the median value of the normospermics at 1 hour was 30.8 μm/sec. Unfortunately, no fertile control group was identified in this paper and one can only come to the conclusion that those samples displaying better qualities in all parameters would naturally have a tendency to have a higher initial sperm velocity with a smaller decline in this parameter at three and five hours.

In Janick & Macleod's paper (1970) sperm velocity

measurements achieved were again compared to a progression parameter from + to ++++. Again the dividing line between ++ and +++ appeared to correlate with a sperm velocity of around 30μm/sec.

Makler in 1979c reported on the sperm velocities measured by MEP in 100 fertile men, although no definition of fertility was offered. The average speed of spermatozoa in this series was 30.3μm/sec, but the velocities were measured in undiluted semen. Interestingly, the mean sperm count in this series was 66 million/ml with a standard deviation of 47 million/ml and the % motility was 45% with a standard deviation of 18.2%. As yet he has not presented any figures concerning the correlation of sperm velocity (as measured by his method) and fertility.

Certainly the figures from this series suggest that sperm velocity as measured by TLP will become a useful clinical tool in the assessment of a patient's eventual fertility. It is certainly interesting looking at Figure 3.2 which shows the "corrected" mean velocities in the fertile and infertile groups, that apart from two patients, 30μm/sec still appears to be the significant level of fertility.

Final Conclusions:

Time-lapse photography is a reliable method of assessing sperm velocity with a coefficient of variation of 4 - 8%. Its accuracy is limited at low density levels - i.e. less than 5 million/ml.

Sperm velocity is a separate parameter from percentage motility and sperm density. It is not dependent on the hormonal serum levels which were measured in this series.

There is a significant difference between the sperm velocities (TLP) between an infertile and fertile group. The method is therefore suggested as an important new diagnostic test in the evaluation of fertility in male patients.

PART II

THE ROLE OF PROLACTIN IN SPERMATOGENESIS

CHAPTER V

POSSIBLE ROLE OF PROLACTIN IN SPERMATOGENESIS

- A Review of the Literature

POSSIBLE ROLE OF PROLACTIN IN SPERMATOGENESIS

(a) Serum Prolactin:

The possible role of prolactin in human reproduction is becoming increasingly important. Hyperprolactinaemia in the female is related to reproductive disturbances such as amenorrhoea (Seppala 1975, 1976 a), the amenorrhoea-galactorrhoea syndrome (Friesen 1973a), anovulatory cycles (Bohnet 1975) and luteal insufficiency (Seppala 1976b).

Human prolactin was first isolated by Bryant et al (1971). They showed that it was a separate entity from growth hormone. Initially studies concentrated on the female gonadal function. The possibility that raised serum prolactin might also interfere with male gonadal function was first raised in 1974 by Thorner et al who reported four cases of raised prolactin levels coupled with galactorrhoea and hypogonadism. Three of the four patients were found to have a micro-adenoma of the pituitary. All these patients presented with impotence. This was successfully treated by administering Bromocriptine to lower the serum prolactin. Unfortunately, no results of seminal analysis were reported in this paper.

A group headed by Anil Sheth working in Bombay (1975) set out to determine the presence of prolactin in human semen and to determining whether the level of the hormone was related to the

quality of semen. Prolactin at this time had already been reported in amniotic fluid, milk and pituitary gland and blood (Friesen,1973 b and Frantz, 1973). Several other reports had indicated that prolactin might be involved in the growth and maintenance of the testis and the accessory reproductive organs of the male rat (Negro-Vilar 1973). Sheth's paper confirmed the presence of prolactin in human semen using radioimmunoassay procedures and that the levels of prolactin were higher in semen samples having a good spermatozoal count and motility. This was the first suggestion that human prolactin might be connected with male fertility.

The following month Sheth (1976 reported further information on the effect of prolactin on the metabolism of human spermatozoa. They investigated the effect of prolactin on cyclic AMP accumulation, fructose utilisation and glucose oxidation by human spermatozoa. The data reported revealed that the physiological amounts of prolactin in semen gave rise to an increase in AMP formation by the human spermatozoa and also stimulated cellular fructolysis. They suggested that enhancement by prolactin of spermatozoal fructose utilisation and glucose oxidation was probably mediated by an increase in cyclic AMP production. They finally suggested that prolactin might play an important part in sperm physiology, especially as related to the transport storage survival.

Segal (1976) and workers in Israel, looked at the association of prolactin with defective spermatogenesis in humans. They stressed that interaction between gonadotrophins and prolactin along the hypothalamic

pituitary testis axis might be important for normal spermatogenesis, since prolactin-producing tumours in rats have induced hypogonadism. In this paper they measured the serum prolactin levels in 25 fertile and 127 infertile males who were again subdivided into oligospermia, azoospermia and hypogonadism. The quality of the fertile group and definition however, was not described. The duration of infertility of the patients selected ranged from 2 - 8 years and had already been screened to ensure normal sella turcica, visual fields and optic fundi. Semen analysis was carried out once weekly for four weeks following a period of four days' abstinence. One or two blood samples were obtained from the ante-cubital vein. Serum was measured for prolactin, follicle-stimulating hormone and luteinizing hormone levels by radioimmunoassay.

They concluded that serum gonadotrophin and prolactin levels in infertile men showed no direct interaction. They suggested that such an interference might exist at testicular level. It was noted that all the hyperprolactinaemic patients had a sperm count of less than 20 million/ml which suggested some interference with spermatogenesis, previously postulated by Sheth (1975).

They then administered Bromocriptine 2.5 mg twice daily for ten weeks to two hyperprolactinaemic patients with oligospermia and hypogonadism. These patients showed a subsequent increase in sperm density and they suggested that this improvement was solely due to the suppression of the prolactin. It must be stressed however, that

this was an extremely small group and that no pregnancy was achieved.

The suggestion that Bromocriptine might help in such cases produced a group of workers in Iran, working under Saidi (1977) to test the possibility of using this drug in the treatment of male infertility. They reported on a group of 11 patients - 8 oligospermic (sperm density - up to 8.4×10^6) and 3 azoospermic. All presented with slightly raised prolactin levels. They were all given Bromocriptine 2.5 mg b.d., but unfortunately three showed a severe intolerance to the drug and treatment had to be stopped. In the remaining five cases, four showed an increased sperm density, within twenty days of the onset of treatment and in all cases a 5 - 10-fold increase in sperm count occurred within four to seven weeks. The improved testicular function was also demonstrated by an equally rapid rise in the serum testosterone level where this had been depressed. However, they did stress that the three patients who started with a normal serum testosterone showed a smaller increase, and that the raised levels of FSH and LH usually observed before treatment, were sometimes maintained for the first few weeks, but then tended to fall progressively. Confirmation of the improved fertility status of these five patients came when three pregnancies were recorded during the same months as the gonadal function returned to normal.

The three azoospermic patients showed similar hormonal responses with striking improvement in testosterone production, but not one, even after prolonged course of treatment, showed any recovery.

to sperm production. He contended that these results, despite the very small series and the lack of control group, suggested that Bromocriptine induced lowering of serum prolactin allowed circulating gonadotrophins to act on the gonads, stimulating steroidogenesis and promoting a return to normal gonadal function. The increasing steroidogenesis may then facilitate a sex steroid feedback on the hypothalamus and pituitary, allowing a return to more normal patterns of gonadotrophin release (Schally, 1972 and Damassa, 1976). They did note that the effect of Bromocriptine was almost immediate and caused the relief of the prolactin-induced blockade of the action of gonadotrophins on the gonad.

This apparent connection between plasma prolactin levels and androgen metabolism , was further investigated by Magrini (1976) in Switzerland. He examined a group of six normal men who on four consecutive days were treated with Sulpiride which is known to raise serum prolactin . The effect of the treatment on testosterone and dihydrotestosterone was examined before and after administration of HCG in this group and a similar group not treated with Sulpiride (with normal prolactin levels). In the controls, the parallel rise in testosterone and dihydrotestosterone was observed in response to HCG stimulation. However, in the experimental hyperprolactinaemic group, the rise in testosterone in response to HCG was accompanied by a markedly diminished rise in dihydrotestosterone. The same results were observed in another patient with hyperprolactinaemia, following apparent accidental section of pituitary stalk. It suggested that thesedata demonstrated the interference of increased levels of prolactin in the metabolism of

testosterone into the active dihydrotestosterone form by 5α reductase. This interference in the conversion of testosterone to dihydrotestosterone, which is the most powerful androgen in the seminiferous tubule, could possibly disturb spermatogenesis throughout its course and the natural reduction in the number of spermatids would result in a fall in inhibin levels. This possibly could explain the marginally raised FSH and LH levels found in these patients. However, he concluded this following the observation that the sleep-related increase of plasma testosterone appeared to be related not only to serum luteinizing hormone concentrations, but also to prolactin levels as well. Rubin (1976) suggested that the prolactin - far from blocking the Leydig function, might possibly stimulate it.

By 1976 two main hypotheses persisted concerning the role of human prolactin in spermatogenesis. The first was that the raised prolactin levels blocked the binding of gonadotrophins to the gonad while the second suggested an interference in the conversion of testosterone to dihydrotestosterone by 5 α reductase.

Pierrepoint (1978) stated:-

> "prolactin remains something of an enigma in men, although now considerable evidence has been found which shows profound action both on the testis and accessory sex glands in experimental animals".

He examined the connection between prolactin and testosterone levels in the plasma of fertile and infertile males. The fertile group

were requesting vasectomy; unfortunately the time since the last episode of fertility was not stated. The infertile group had a density range from 5 - 30 x 10^6 with a median of 9.9 x 10^6 and had been unsuccessful over two years in impregnating their wives. Again, the criteria of male selection was done on the basis of exclusion in the female. In the fertile group the density count ranged from 8.8 million to 272 million with a median of 64 million.

Levels of prolactin and testosterone in the plasma of fertile and infertile men (means, ± S.D.)

	Fertile	Infertile	
		Oligospermia	Azoospermia
Testosterone (ng/100ml)	649.6 ± 181.51 (33)	525.4 ± 138.55*** (84)	534.5 ± 195.46** (49)
Prolactin (ng/l)	0.22 ± 0.16 (27)	0.14 ± 0.10*** (94)	0.16 ± 0.10* (57)

The figures in parenthesis indicate the number of patients.

*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, compared with the levels of the fertile group as assessed by the Mann-Whitney U test.

Table 5.1

Examination of the results reveals that the concentrations of both testosterone and prolactin were significantly lower in the infertile as compared to the fertile men. Again they seemed to stress

the correlation between testosterone levels and prolactin.

Whereas the previous workers had shown higher levels of prolactin in subfertile males, the combined lowering of prolactin and testosterone in the infertile men caused Pierrepoint to speculate that in concert with other hormones, deficiency in prolactin is as detrimental to the individual as an excess.

One further paper appeared by Koskimies (1978) looking at both serum and seminal plasma prolactin levels in oligospermia. In this paper, 26 oligospermic and 23 normospermic men were studied. All had normal libido, signs of virility and no endocrinological or other diseases were found. Their definition of oligospermia was taken as a density count of $<$ 40 million. The fertility status of the normospermic group was not stated.

They showed that serum prolactin concentration in the normospermic male was 24.5 $\pm$ 1.6 ng/ml. In the oligospermic group the means serum prolactin concentration was 25.3 $\pm$ 1.5 ng/ml. No significant difference was found in the mean serum prolactin concentrations, between oligospermic and normospermic groups. However, correlation analysis within oligospermic men revealed an inverse correlation ($p = < 0.005$) between serum prolactin concentration and sperm count. They suggested that from this information serum prolactin was involved in the regulation of spermatogenesis in man.

They ended their paper with one very pertinent remark:-

"these results together with recent remarks concerning Bromocriptine effect upon sperm count, are encouraging enough to warrant controlled trials on the suppression of prolactin secretion in the treatment of male infertility".

After initial excitement, later reports on the role of hyperprolactinaemia in male infertility and spermatogenesis have not been so encouraging; (Carter et al (1978), Jequier et al (1979), Rjosk & Schill (1979). In the latter, the range of serum prolactin measured in normospermic patients varied from 80 to 350 mU/ml. This was in accordance with the Results of both Sheth (1973) and Segal (1976). Rjosk found elevated serum prolactin levels in two of seventy-one patients presenting with oligospermia and asthenospermia respectively. In one patient the prolactin levels were observed during treatment with amitryptiline which was thought to be the cause. The other patient - no cause for the elevated prolactin level was found. The incidence of hyperprolactinaemia in this paper was similar to the 4% found in Segal (1976) and 9% reported by Roulier et al in 1978. However, he did conclude that the role of hyperprolatinaemia on male infertility is still poorly understood and suggested the reports on the restoration of male infertility by the treatment of Bromocriptine should be interpreted with caution. When comparing the normospermic with oligospermic patients in his paper, Rjosk found no significant difference in serum prolactin levels, confirming the results of Sheth (1973). Also he agreed with Sheth (1973), Segal (1976) that no correlation persists between serum prolactin levels with LH, FSH, testosterone, sperm count and sperm motility.

There was however, a weak negative correlation between serum prolactin and testosterone within the oligospermic patients, but doubted whether this was of biological relevance.

Further evidence for a possible role of prolactin in spermatogenesis came with the isolation of prolactin receptors in the Leydig cells (Aragona, 1977), Charreau (1977). These receptors are specific for prolactin and LH does not compete with this hormone.

Finally, an interesting concept was suggested by Fraioli (1980) who reported the case of a 23 year old man presenting with a pituitary prolactin-secreting adenoma, where all other endocrinological parameters were normal. Whereas seminal analysis had shown normal spermatogenesis, electron microscopy studies of the testicular specimen revealed the presence of undivided spermatids, containing two or more nucleii, partially embedded in a developing common acrosome. This morphological picture was demonstrated on constant "paired" situation. Following treatment with 5 mg per day Bromocriptine, prolactin levels decreased and this particular abnormality disappeared. From this information, he speculated that prolactin acts positively on the final maturation process of spermatozoa, either directly or through an intermediate growth factor.

(b) Seminal Prolactin Levels:

It has already been suggested in the previous section that the interference of prolactin in spermatogenesis might well be at seminal fluid level. In one of the earlier papers on this subject by Sheth et al (1975) entitled: Occurrence of Prolactin in Human Semen - it was shown conclusively that prolactin was present in seminal fluid. It also went on to show that the levels were 4 to 7 fold higher than those reported in the serum of fertile and infertile men. At that time they stated the most likely sources of prolactin in human semen are from secretions in the prostate and seminal vesicles. At that time it stated that the mechanisms which control the secretion of prolactin in the human were not clearly understood.

Boyns (1973) studied the prolactin levels of normal men and those with prostatic cancer and found that there is a better correlation between prolactin and testosterone levels than between luteinizing hormone and testosterone. This indicates that prolactin might also stimulate testosterone production in men. In view of this, Sheth et al (1976) speculated on the possible role of prolactin in the testis and the accessory reproductive glands.

A review by Nicholl & Burn (1972) listed 82 possible functions of prolactin in difference animal species. Some of these involved water and electrolyte metabolism and suggested that further consideration was required to understand the possible physiological role of the seminal prolactin. They

went on to state that sperm motility and metabolism are greatly influenced by the ionic concentrations in the surrounding medium and suggested prolactin may influence the sodium and potassium transport between spermatozoa and the seminal plasma. They also considered that it would be worthwhile to determine how prolactin affects the oxidative or carbohydrate mechanism of the spermatozoa.

Shah et al (1976) observed that the utilization of fructose, the oxidisation of glucose and the accumulation of cyclic adenosine 3':5' monophosphate by normal human spermatozoa was stimulated by the presence of prolactin. Pedron in 1978 looked at the effect of prolactin on the glycolytic metabolism of spermatozoa of married infertile men whose semen showed spermatozoal concentrations of less than 40 million/ml. In this paper he showed that in spite of having an increased glycolytic metabolism, spermatozoa from oligospermic semen are able to utilise additional glucose when subjected to the hormonal stimulus of prolactin. This type of response indicates that phosphofructokinase can be activated by prolactin. Later, Sheth (1979) looked at the effect of LH and prolactin in human semen on spermatozoal ATPase activity. The basis of this investigation was the fact that the motility of spermatozoa is dependent upon the rate that energy is immediately available by the enzymic breakdown of ATP (Mitchell, 1976). They showed that both prolactin and LH enhance the activity of ATPase, suggesting that these hormones, by their presence in seminal fluid, may influence the energy metabolism of spermatozoa. This is an important concept, since prolactin and LH are known to affect

sperm ATPase. There is also a known association of ATPase with sperm capacitation (Gordon, 1977), and this brings up the possibility that prolactin at seminal level may have some effect upon capacitation.

Koskimies in 1978 reported the following data on seminal prolactin levels:

In their normospermic group - i.e. men with a sperm density greater than 40 million/ml, the seminal prolactin level was 133.6 $\pm$ 9.9 ng/ml, while in the oligospermic group it was 119.3 $\pm$ 12.3 ng/ml. This difference was not significant. Unlike the correlation found within the oligospermic group between serum prolactin and sperm count, no such correlation was found between seminal prolactin levels and sperm density.

Smith (1979) reported on semen samples collected from 50 prevasectomy patients and 43 infertility clinic patients. He found a significant correlation between the semen prolactin concentration and sperm count for both the prevasectomy ($r = 0.73$) and the clinic patients ($r = 0.72$). He also found a correlation between the total amount of prolactin per ejaculate and sperm motility ($r = 0.46$) and between ejaculatory volume and percentage motility ($r = 0.36$). The latter work confirmed the work of Sheth (1975) and Biswas (1978).

At the time of writing (1980) there seems to be conflicting evidence as to whether hyperprolactinaemia at a plasma level interferes with spermatogenesis. The initial

reports have recently been disproved. At a seminal level however, prolactin appears to have some effect on spermatogenesis and sperm motility, though whether this is a primary effect or secondary to an interference of androgen function, is yet to be determined.

CHAPTER VI

MATERIALS AND METHODS

PATIENT SELECTION

Selection Criteria for Inclusion in Infertile Group:

Males were recruited voluntarily from the Southampton Infertility Clinic. Informed consent was given. The criteria for selection was as follows:

(a) Exclusion of female factor:

The following criteria were considered:

Both multiparous and nulliparous female partners were accepted. Any female with a past history of chronic pelvic infection was excluded, as well as those who had taken oral contraceptive up to two years prior to the start of investigations. A careful general examination was carried out to exclude any major medical disorders. A vaginal examination was carried out, paying particular attention to the cervix, the size, motility, position of the uterus and normality of both adnexae. Finally, a careful assessment of suitable cervical mucus was carried out between the days 12 - 14. Unfavourable mucus at this point excluded the partner from the trial. Special investigations included evidence of tubal patency with dye insufflation at laparoscopy. Any signs of old pelvic infection in the form of adhesions or visual abnormality of ovaries excluded the patient from the investigation.

The following serum investigations were also carried out on the female:

(i) T3 and T4.

(ii) Serum prolactin (levels up to 380 mIU/l were accepted as normal).

(iii) Plasma progesterone taken on day 21 by venepuncture on three consecutive cycles. All results had to be in excess of 30 nmol/l in order to show adequate luteal function. Intra-assay variation was assessed.

(b) Criteria for selection of the infertile male:

If the female partner had satisfied the above criteria, the following criteria were applied to the male partner:

A careful general examination was carried out to exclude any major medical disorders. Careful examination of the scrotum was carried out to exclude varicocele, congenital absence of vas and hypoplastic testicles (i.e. less than 4 ccs).

An initial seminal analysis was carried out. No particular density count was chosen, although a low limit of 5 million/ml was established, due to the experience of methodology of sperm velocity assessment (discussed in Part I - Chapter II). Again, it must be stressed that in view of the referral system to the Southampton Male Infertility Clinic, based upon the diagnosis of oligospermia (i.e. $< 40 \times 10^6$/ml) there was a bias on the density count of the spermatozoa of gentlemen presenting.

At initial assessment, a sample was produced by masturbation into a sterile plastic container and reviewed visually after half an hour when liquefaction must have taken place for inclusion in the trial. The ejaculate was carefully studied for any signs of genital infection, such as white cells or bacteria and also sent for culture. Any signs of infection excluded the patient from the trial.

Fifty such couples were screened for the above criteria and having satisfied the requirements, were entered into the investigation.

Selection criteria for inclusion in the fertile group.

Twenty males were recruited voluntarily. The criteria for inclusion in the fertile group were the same as in Chapter II

SERUM MEASUREMENTS

(a) Prolactin:

Serum prolactin was kindly measured by Dr. Anne Evans in the Department of Chemical Pathology, Southampton General Hospital, by a radioimmunoassay using sheep antisera to human prolactin. This was kindly provided by Dr. Graham Groom, Tenovus Institute, Cardiff. The radioactive antigen was obtained from Dr. P. G. Lowry, Department of Chemical Pathology, St. Bartholomew's Hospital, London. A laboratory standard was used which was calibrated to the first International reference preparation - code no: 75/504 and this assay was part of the National quality-control scheme. A double antibody method of separation was used which achieved a 95% separation.
Six quality-controlled samples were added to each assay and the intra-assay coefficient of variation was 2.8% and the inter-assay coefficient of variation was 4.8%.

(b) 17B Oestradiol, FSH, LH were measured using the same methodology as described in Part I Chapter III.

SEMINAL FLUID MEASUREMENTS

(a) Prolactin:

Seminal prolactin was also measured by a similar radioimmunoassay by Dr. Anne Evans, Department of Chemical Pathology, Southampton General Hospital. The methodology was similar to that described under Serum Prolactin. The same International reference preparation code no: 75/504 was used and despite most of the readings being at the top end of the standard curve, the intra-assay and inter-assay coefficients of variation were the same as for Serum Prolactin.

(b) Fructose and Myoinositol:

Fructose and Myoinositol were measured by the same methodology as described in Part I Chapter III.

DESIGN OF STUDY

Fifty males who had satisfied the above requirements were entered into the study and asked to attend the University on two occasions separated by two weeks. At each visit they were required to abstain from sexual intercourse for four days prior to sampling. 20 mls of blood had been removed by venepuncture, placed in sterile bottles and allowed to clot. The serum was separated, labelled and stored in aliquots at -20°C.

Seminal samples were produced by masturbation. At the time of ejaculation a timer was started and as described in Section A, sperm velocity measurements were taken exactly thirty minutes later, using time-lapse photography (TLP). Following sperm velocity measurement and semen analysis (see Part II) the seminal fluid was spun at 3,000 r.p.m.for twenty minutes, the fluid was removed and aliquoted and stored at -20°C for future evaluation.

At the second visit the above procedure was repeated. Each male was started on a regime of tablets for six months. The trial was set up as a parallel group study. The patients received either Bromocriptine 2.5 mg or placebo on a double-blind basis. All males

were given the same instructions as to the administration of the tablets: they were to be taken initially after food in the evening starting at half a tablet (1.25 mg) and increasing in 1.25 mg steps in the absence of any side-effects. All males were taking the full dosage of 5 mg in the evening of either Bromocriptine or placebo within one month of starting the trial.

The patients were then seen at three months and six months when a similar procedure was carried out as already stated. Prior to sampling all patients were carefully questioned on any side-effects. These were noted. Again samples, both serum and seminal fluid were produced in a similar manner, assessed and stored with suitable coding at $-20^{\circ}C$. Following completion of the trial, the investigators were supplied with the information concerning the identity of the placebo group.

During the six months on treatment, the wives of all patients had serum progesterone carried out on day 21 on three occasions, in order to establish continued adequate luteal function. In the cases where pregnancy took place, the male continued on his dosage regime to the full six months.

CHAPTER VII

RESULTS

(a) SERUM PROLACTIN:

The mean serum prolactin in the fertile group (20) at visits (a) and (b) was 219.9 mIU/1 with a standard deviation of 97.5 mIU/1 (see Table 3.15). The mean serum prolactin of the infertile group (50) at visits (a) and (b) was 220.1 mIU/1 with a standard deviation of 84.4 mIU/1. There was no statistical difference between the two groups ($p = 0.98$). There was also no statistical difference between the two groups when considering visits (a) and (b) separately ($p = 0.98$ and 0.95 respectively). In the fertile group there was no statistical difference between the serum prolactin levels between the two visits (a) and (b) ($p = 0.35$). A similar situation was found in the infertile group ($p = 0.21$).

CORRELATION WITH OTHER PARAMETERS:

The same parameters were studied and correlated both in the fertile (20) and in the infertile (50) groups. This included all the parameters measured at the seminal analysis and the serum hormonal estimations as described in Part I.

Fertile Group:

As can be seen in Table 7.1 there were no statistical correlations between serum prolactin in any parameter of seminal analysis or in any serum hormonal investigations at the 5% level.

Infertile Group:

The mean serum prolactin of the infertile group was taken and statistically correlated with the other parameters as described. There was no statistical correlation found with the following parameters:

v s. Velocity	($p = 0.07$)
v s. Percentage Dead Cells	($p = 0.49$)
v s. Percentage Motility	($p = 0.40$)
v s. Percentage Live Motility	($p = 0.39$)

There was a statistical correlation between serum prolactin and percentage abnormal forms ($p = 0.017$). In contrast, there was no correlation with sperm density ($p = 0.43$) but there was a negative correlation with the total number of sperm present in each ejaculate ($p = 0.04$). A similar negative correlation was found between serum prolactin and total number of live sperm ($p = 0.04$) and also with the total number of motile spermatozoa in the ejaculate ($p = 0.05$).

	Serum Prolactin				Seminal Prolactin			
	FERTILE		INFERTILE		FERTILE		INFERTILE	
	r =	p =	r =	p =	r =	p =	r =	p =
Velocity	0.08	0.30	0.14	0.07	0.23	0.07	0.11	0.14
% Dead	0.03	0.40	-0.001	0.49	0.03	0.41	0.24	0.006
% Motility	-0.07	0.31	-0.02	0.40	-0.04	0.39	0.05	0.29
% Live Motility	-0.05	0.36	-0.02	0.39	-0.04	0.39	0.18	0.03
% Abnormal Forms	0.11	0.24	0.21	0.017	-0.23	0.08	0.12	0.12
Density	-0.08	0.31	-0.01	0.43	0.03	0.42	0.12	0.12
Total Nos. of Spermatozoa	-0.02	0.43	-0.17	0.04	0.02	0.44	0.003	0.48
Total Live Spermatozoa	-0.04	0.38	-0.17	0.04	0.03	0.42	-0.03	0.40
Total Motile Spermatozoa	-0.03	0.41	-0.16	0.053	0.03	0.43	0.01	0.44
Total Normal Spermatozoa	-0.05	0.37	-0.19	0.027	0.07	0.31	-0.01	0.45
Seminal Volume	0.14	0.19	-0.19	0.028	-0.03	0.41	-0.14	0.079

Table 7.1: CORRELATIONS (r =) AND SIGNIFICANCE (p =) IN FERTILE AND INFERTILE PATIENTS OF SEMINAL AND SERUM PROLACTIN TO THE PARAMETERS OF SEMINAL ANALYSIS.

There was also a negative correlation between serum prolactin and the total number of normal spermatozoa in the ejaculate ($p = 0.027$). Seminal volume negatively correlated with serum prolactin ($p = 0.028$).

When considering the serum hormonal estimations carried out, there was no correlation of serum prolactin with the following parameters in the infertile group (see Table 7.2):

17β Oestradiol	($p = 0.23$)
LH	($p = 0.24$)
DHT	($p = 0.32$)

There was a significant statistical correlation between serum prolactin and follicle stimulating hormone ($p = 0.01$).

In order to check the validity of the above correlations, the infertile group (50) was further sub-divided into two groups according to whether their serum prolactin level was above and below the mean serum level of the infertile group (220 mIU/1). Separation produced thirty-eight samples of above the mean level of 220 and 62 samples below the mean level of 220. These two groups were then compared statistically and in spite of the skewed distribution, differences were found as enumerated above. In the group above 220 mIU/L there were statistically higher levels of abnormal forms ($p = 0.018$), lower levels of a total number of spermatozoa ($p = 0.012$), lower levels of the total number of live spermatozoa ($p = 0.013$), lower numbers of total motile spermatozoa ($p = 0.015$), lower numbers of the total number of normal spermatozoa ($p = 0.005$). This group also had a significantly lower seminal volume than the group with the serum levels below 220 mIU/L. ($p = 0.05$).

	FERTILE (20)		INFERTILE (50)	
	r =	p =	r =	p =
Oestradiol	0.01	0.47	0.07	0.23
LH	0.19	0.11	0.07	0.24
FSH	0.07	0.32	0.23	0.01
DHT	-0.03	0.40	0.04	0.32

CORRELATIONS (r =) AND SIGNIFICANCE (p =) OF SERUM PROLACTIN TO SERUM HORMONAL ESTIMATIONS.

Table 7.2

	FERTILE (20)		INFERTILE (50)	
	r =	p =	r =	p =
Oestradiol	0.15	0.17	-0.004	0.48
LH	-0.04	0.39	0.23	0.011
FSH	0.19	0.11	0.26	0.004
DHT	-0.03	0.41	0.11	0.12

CORRELATIONS (r =) AND SIGNIFICANCE (p =) OF SEMINAL PROLACTIN TO SERUM HORMONAL ESTIMATIONS.

Table 7.3

(b) SEMINAL PROLACTIN:

The mean seminal prolactin in the fertile group (20) at visits (a) and (b) was 863.5 mIU/1 with a standard deviation of 168.4 mIU/1 (see Table 10.2(a)).

The mean seminal prolactin of the infertile group (50) at visits (a) and (b) was 828.2 mIU/1 with a standard deviation of 191.5 mIU/1. There was no statistical difference between the two groups ($p = 0.31$).

There was also no statistical difference between the two groups when considering visits (a) and (b) separately ($p = 0.38$ and $p = 0.40$ respectively). Similar to serum prolactin, there was no statistical difference between the seminal prolactin levels between the first and second visits in either the fertile or the infertile group.

CORRELATION WITH OTHER PARAMETERS:

As with serum prolactin the other parameters studied were correlated with seminal prolactin in both the fertile and infertile groups (see Table 7.3).

Fertile Group:

Throughout all parameters both in seminal analysis and in serum hormonal investigations, there was no statistical correlation between these and the seminal prolactin levels at the 5% level. The closest any parameter got to showing a significant correlation to seminal prolactin was sperm velocity ($p = 0.07$).

Infertile Group:

Within the seminal analysis the only significant correlation at the 5% level with seminal prolactin occurred in the following parameters:

Percentage Dead Cells ($p = 0.006$)

Percentage Live Motility ($p = 0.03$).

Both correlations were positive. There was no significant correlation between seminal prolactin and seminal volume ($p = 0.079$).

In the serum hormonal estimations, seminal prolactin did not correlate with oestradiol ($p = 0.48$) or DHT ($p = 0.12$). There was however a strong correlation between seminal prolactin and LH ($p = 0.011$) and FSH ($p = 0.004$), both of a positive nature.

Unlike the fertile group seminal prolactin did strongly correlate positively with serum prolactin ($p = 0.006$).

THE EFFECT OF PROLACTIN LOWERING AGENT (BROMOCRIPTINE) IN FIFTY INFERTILE MALES:

INTRODUCTION:

There was no statistical difference between the two different groups (Bromocriptine treated and placebo treated) at any of the parameters described in Section I. This was true at both (a) and (b) visits separately and when considering the mean of both visits. This lack of correlation between the two groups at any parameter shows that the two groups were equally matched and any ensuing difference found at the levels after treatment will be accurate.

(a) SERUM PROLACTIN:

In Table 7.4 the Bromocriptine group had a significant reduction in serum prolactin levels after three months' treatment to a mean level of 83.6 mIU/1 ($p = 0.0005$). However at six months the mean serum levels had returned to 148.3 mIU/1 and this rise from 3 to 6 months was statistically significant ($p = 0.045$). There was also a statistical difference between the pre-treated levels and the levels at six months ($p = 0.026$). In the placebo treated group no such significant differences were found at either three months or six months ($p = 0.14$ and 0.80 respectively). These results are also shown in Figure 7.1.

Mean levels (± S.D.) of Serum Prolactin (mIU/1) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (mIU/1)	217.0	83.6	148.3
± S.D.	72.6	73.6	141.7
Mean of Placebo Group (n = 24) (mIU/1)	223.6	190.5	217.8
± S.D.	97.0	74.9	82.1

Significant changes (p =) in Serum Prolactin after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.0005	0.026	0.045
Placebo Group (n = 24)	0.14	0.80	0.23

Table 7.4

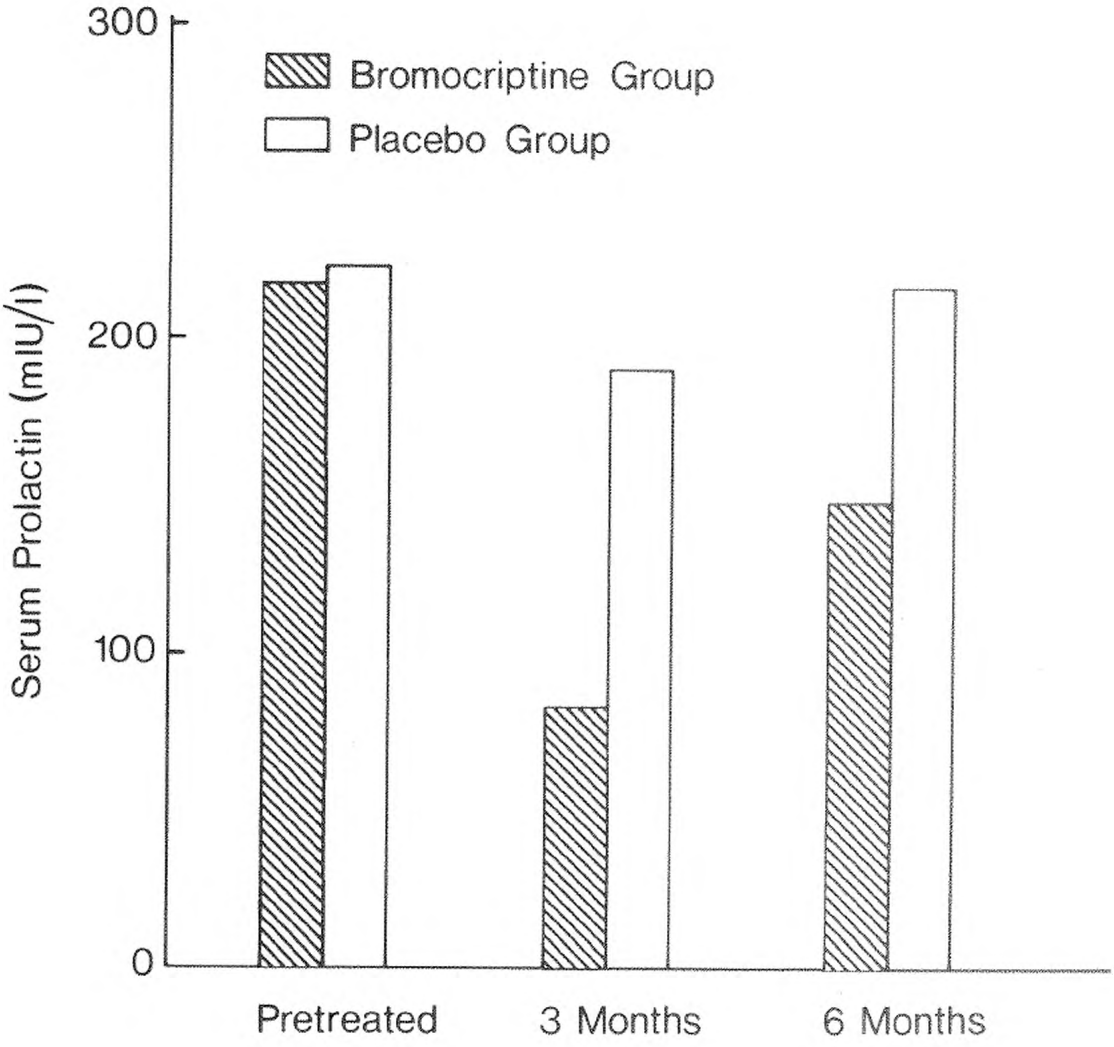


Figure 7.1: Serum Prolactin (mIU/l) after treatment with Bromocriptine or Placebo

PARAMETERS OF SEMINAL ANALYSIS

Sperm Velocity (μm/sec):

With reference to Table 7.5 there was no significant difference in the Bromocriptine treated group at either three or six months as compared to the pre-treated levels ($p = 0.8$ and 0.46 respectively). There was also no significant difference found in the placebo group at three and six months ($p = 0.38$ and 0.94 respectively).

Mean levels (± S.D.) of Sperm Velocity (μm/sec) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (μm/sec)	27.37	27.08	26.47
± S.D.	4.7	5.2	5.49
Mean of Placebo Group (n = 24) (μm/sec)	29.17	27.80	29.28
± S.D.	6.21	6.17	5.58

Significant changes (p =) in Sperm Velocity after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.8	0.46	0.68
Placebo Group (n = 24)	0.38	0.94	0.39

Table 7.5

Percentage Live/Dead:

Table 7.6 shows no statistical difference in the percentage of dead cells found in the Bromocriptine treated group at either three or six months when compared to pre-treatment levels ($p = 0.24$ and 0.81 respectively). A similar situation was found in the placebo group ($p = 0.29$ and 0.64 respectively).

Mean levels (± S.D.) of Percentage Dead Cells (%) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (%)	16.1	14. 2	15.8
± S.D.	6.8	7.1	10.5
Mean of Placebo Group (n = 24) (%)	17.3	15.5	18.4
± S.D.	7.5	5.6	10.5

Significant changes (p =) in Percentage Dead Cells after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.24	0.81	0.58
Placebo Group (n = 24)	0.29	0.64	0.23

Table 7.6

Percentage Motility:

In Table 7.7 there was no statistical difference in the Bromocriptine treated group at three and six months when compared to pre-treatment levels ($p = 0.82$ and 0.07 respectively). The same situation was found in the placebo treated group ($p = 0.95$ and 0.71 respectively).

Mean levels (± S.D.) of Percentage Motility (%) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (%)	61.2	60.6	56.5
± S.D.	9.6	12.3	12.3
Mean of Placebo Group (n = 24)	61.7	61.8	60.7
± S.D.	10.6	9.7	9.8

Significant changes (p =) in Percentage Motility after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.82	0.07	0.23
Placebo Group (n = 24)	0.95	0.71	0.69

Table 7.7

Percentage Live Motility:

Percentage live motility progressively fell throughout treatment with Bromocriptine. Although the levels at three months were not significantly different from pre-treatment levels ($p = 0.28$), by six months, a definite statistical significance had appeared ($p = 0.016$). No such effect was noted in the placebo treated group throughout the six months' treatment. (See Table 7.8).

Mean levels (± S.D.) of Percentage Live Motility (%) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (%)	73.2	70.4	66.8
± S.D.	10.3	12.5	11.5
Mean of Placebo Group (n = 24) (%)	74.6	73 .6	74.8
± S.D.	12.6	11.1	11.1

Significant changes (p =) in Live Motility after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.30	0.016	0.28
Placebo Group (n = 24)	0.728	0.96	0.71

Table 7.8

Percentage Abnormal Forms:

Table 7.9 shows that in the Bromocriptine group there was a progressive increase in the percentage abnormal forms in each ejaculate. The level at six months was statistically significant to that before treatment ($p = 0.001$). In the placebo group a similar situation occurred with a progressive increase in the percentage of abnormal forms. When comparing the levels at six months to pre-treatment levels there was also a statistical difference ($p = 0.0005$).

Mean levels (± S.D.) of Percentage Abnormal Forms (%) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26)(%)	41.9	48.9	58.0
± S.D.	19.7	17.2	16.3
Mean of Placebo Group (n = 24)(%)	36.0	44.9	51.6
± S.D.	17.0	17.2	13.8

Significant changes (p =) in Percentage Abnormal Forms after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.13	0.001	0.057
Placebo Group (n = 24)	0.043	0.0005	0.144

Table 7.9

Density (million/ml):

With reference to Table 7.10 in the Bromocriptine group there was no statistical difference in the density (million/ml) counts taken at three and six months when compared to pre-treatment levels ($p = 0.86$ and 0.34 respectively). In the placebo treated group a similar situation was found ($p = 0.30$ and 0.38 respectively).

Mean levels (± S.D.) of Sperm Density ($x10^6$/ml) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) ($x10^6$/ml)	38	39	30
± S.D.	34	39	26
Mean of Placebo Group (n = 24) ($x10^6$/ml)	42	33	34
± S.D.	35	26	33

Significant changes (p =) in Sperm Density after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.86	0.34	0.35
Placebo Group (n = 24)	0.30	0.38	0.92

Table 7.10

Total Number of Spermatozoa Per Ejaculate (millions):

In Table 7.11 the total number of spermatozoa in the ejaculate appeared to fall slightly in the Bromocriptine treated group throughout the six months, although this fall was not statistically significant ($p = 0.19$). In the placebo group the levels appeared to fall at three months but returned to the pre-treatment levels at six months. There was no statistical difference at three months compared to the pre-treatment levels ($p = 0.26$) or between the three and six months level ($p = 0.51$).

Mean levels (± S.D.) of Total Number of Spermatozoa (millions) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (millions)	119	102	89
± S.D.	106	107	75
Mean of Placebo Group (n = 24) (millions)	136	99	126
± S.D.	143	107	160

Significant changes (p =) in Total Number of Spermatozoa after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.51	0.19	0.59
Placebo Group (n = 24)	0.26	0.78	0.51

Table 7.11

Total Number of Live Spermatozoa in the Ejaculate (millions):

With reference to Table 7.12 there appears to be a progressive fall in the levels of this parameter in the Bromocriptine treated group both at three and six months. These falls were not statistically significant ($p = 0.60$ and 0.20 respectively). In the placebo treated group there was also a fall in the total number of live spermatozoa at three months but recovered by six months. There was no statistical difference between any of these levels ($p = 0.29$, 0.67 and 0.56 respectively).

Mean levels (± S.D.) of Total Number of Live Spermatozoa (millions) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (millions)	119	106	74
± S.D.	102	107	66
Mean of Placebo Group (n = 24) (millions)	114	84	96
± S.D.	124	87	112

Significant changes (p =) in Total Number of Live Spermatozoa after tr eatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.60	0.20	0.53
Placebo Group (n = 24)	0.29	0.56	0.67

Table 7.12

Total Number of Motile Spermatozoa Per Ejaculate (millions):

With reference to Table 7.13 in the Bromocriptine group there was a progressive fall in the total number of motile spermatozoa during the six months treatment, although the difference in levels was not significant at either three months ($p = 0.33$) or six months ($p = 0.12$). A similar fall in levels in this parameter was found in the placebo group which is also not significant at either three or six months ($p = 0.29$ and 0.55 respectively).

Mean levels (± S.D.) of Total Number of Motile Spermatozoa (millions) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (millions)	71	58	50
± S.D.	60	51	48
Mean of Placebo Group (n = 24) (millions)	79	61	68
± S.D.	71	61	73

Significant changes (p =) in Total Number of Motile Spermatozoa after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.33	0.12	0.56
Placebo Group (n = 24)	0.29	0.55	0.72

Table 7.13

Total Number of Normal Spermatozoa in the Ejaculate (millions):

In Table 7.14 the Bromocriptine treated group showed a steady fall in the total number of normal spermatozoa progressively over the six months of therapy. The difference in levels at three months as compared to the pre-treatment group was not statistically significant ($p = 0.19$) but at six months there was a statistical difference ($p = 0.007$). In the placebo group there was also a lowering of levels at three months compared to the pre-treatment level although this was not statistically significant at the 5% level ($p = 0.07$). Like the Bromocriptine group there was no statistical difference between the six months and the pre-treatment levels ($p = 0.32$). These results are also shown in the histogram form (Figure 7.2).

Mean levels (± S.D.) of Total Number of Normal Spermatozoa (millions) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26)(millions)	78	55	40
± S.D.	80	61	44
Mean of Placebo Group (n = 24)(millions)	95	57	69
± S.D.	107	64	99

Significant changes (p =) in Total Number of Normal Spermatozoa after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.19	0.007	0.32
Placebo Group (n = 24)	0.07	0.32	0.65

Table 7.14

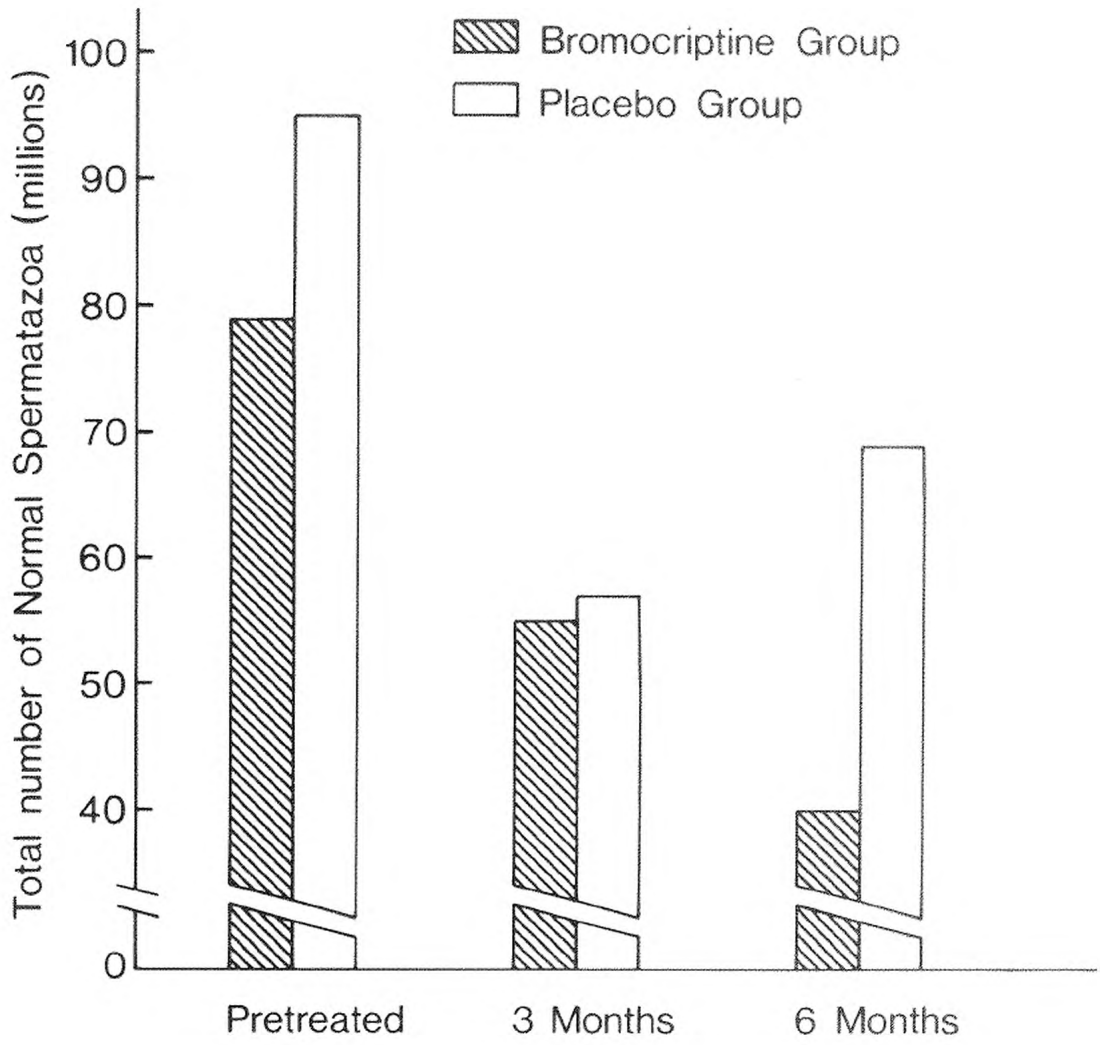


Figure 7.2 : Total number of Normal Spermatazoa (millions) after treatment with Bromocriptine or Placebo

Seminal Volume (ml):

As can be seen in Table 7.15, in the Bromocriptine treated group, there was a reduction in seminal volume after three months' treatment but a return to normal levels by six months. The drop at three months compared to pre-treatment levels was not statistically significant at the 5% level ($p = 0.09$).

Mean levels (± S.D.) of Seminal Volume (ml) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (ml)	3.2	2.7	3.2
± S.D.	1.3	1.2	1.5
Mean of Placebo Group (n = 24) (ml)	3.4	3.2	3.4
± S.D.	1.4	1.5	1.8

Significant changes (p =) in Seminal Volume after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.09	0.79	0.26
Placebo Group (n = 24)	0.46	0.97	0.61

Table 7.15

SERUM HORMONAL ESTIMATIONS

Serum 17β Oestradiol (pmol/l):

As can be seen in Table 7.16, the Bromocriptine treated group showed no statistical difference either at three months or six months compared to pre-treatment levels ($p = 0.56$ and 0.92 respectively) of 17β Oestradiol. In the placebo treated group the same situation was found at both three months and six months ($p = 0.67$ and 0.71 respectively).

Mean levels (± S.D.) of Serum 17β Oestradiol (pmol/l) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (pmol/l)	179.4	170.6	177.1
± S.D.	64.8	61.0	107.5
Mean of Placebo Group (n = 24) (pmol/l)	157.4	167.3	151.3
± S.D.	71.2	102.4	59.3

Significant changes (p =) in Serum 17β Oestradiol after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.56	0.92	0.78
Placebo Group (n = 24)	0.67	0.717	0.51

Table 7.16

Follicle Stimulating Hormone (IU/1):

In Table 7.17 the Bromocriptine treated group showed no alteration in the levels of follicle stimulating hormone at either three months or six months compared to pre-treatment levels ($p = 0.66$ and 0.91 respectively). In the placebo treated group a similar situation was found ($p = 0.37$ and 0.54 respectively).

Mean levels (± S.D.) of Follicle stimulating hormone (IU/1) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (IU/1)	3.5	3.2	3.4
± S.D.	2.7	2.8	2.5
Mean of Placebo Group (n = 24) (IU/1)	3.5	3.0	3.1
± S.D.	2.6	1.9	2.1

Significant changes (p =) in Follicle stimulating hormone after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.66	0.91	0.76
Placebo Group (n = 24)	0.37	0.54	0.77

Table 7.17

Luteinizing Hormone (IU/1):

With reference to Table 7.18 in the Bromocriptine treated group there was a significant reduction in the levels of luteinizing hormone at three months of treatment as compared to the pre-treatment levels ($p = 0.0005$) followed by a statistically significant increase in levels at six months ($p = 0.0003$). The levels at six months were not significantly different from the pre-treatment levels ($p = 0.85$). In the placebo treated group there was also a statistical lowering of luteinizing hormone at three months ($p = 0.005$) followed by a statistically significant increase back to pre-treatment levels at six months ($p = 0.0005$). There was no significant difference between pre-treatment levels and levels at six months ($p = 0.43$). These results are expressed in histogram form in Figure 7.3.

Mean levels (± S.D.) of Luteinizing Hormone (IU/1) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (IU/1)	5.4	3.3	5.5
± S.D.	2.1	2.5	2.4
Mean of Placebo Group (n = 24) (IU/1)	4.6	2.4	4.3
± S.D.	1.6	1.2	1.4

Significant changes (p =) in Luteinizing Hormone after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.0005	0.85	0.003
Placebo Group (n = 24)	0.0005	0.43	0.0005

Table 7.18

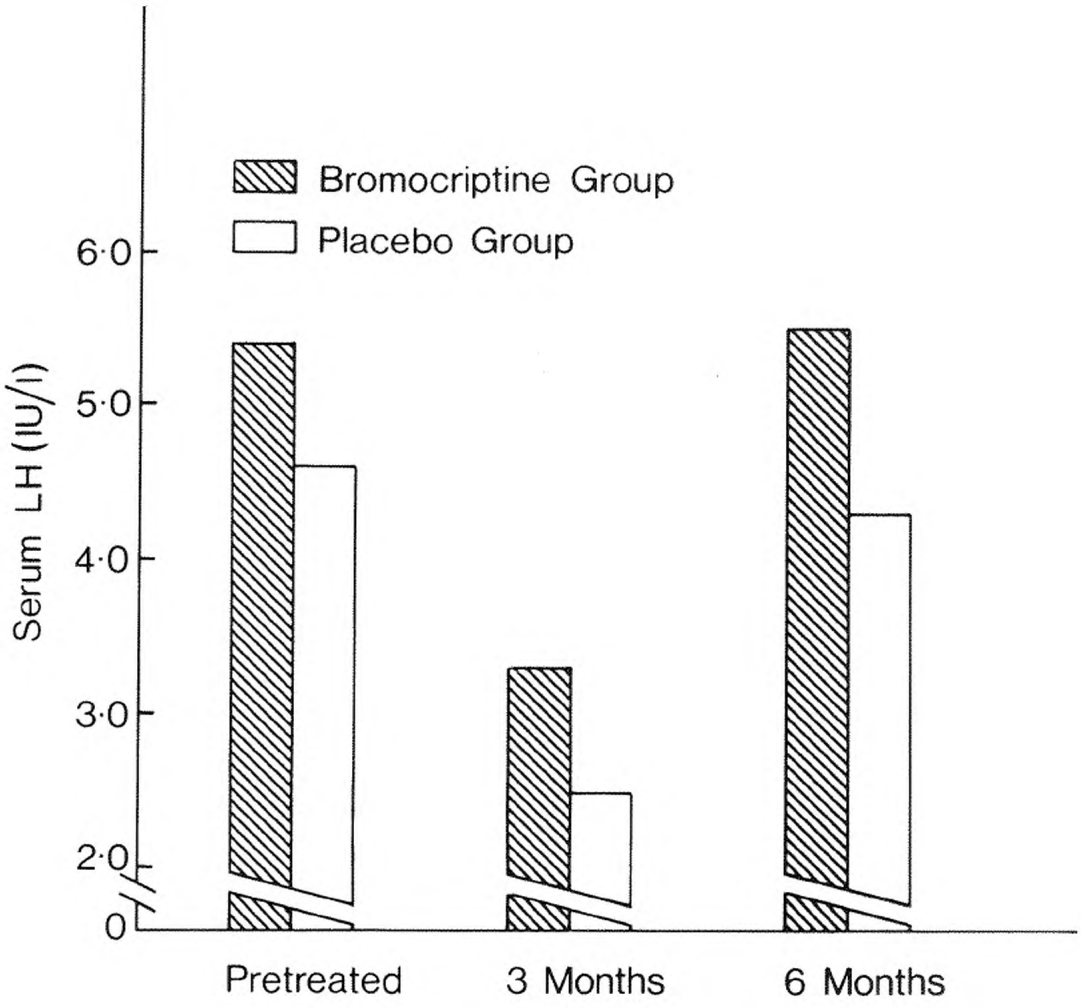


Figure 7.3: Serum LH (IU/l) after treatment with Bromocriptine or Placebo

Dihydrotestosterone (nmol/l):

With reference to Table 7.19 in the Bromocriptine treated group there was a progressive increase throughout the six months in levels of dihydrotestosterone. A significant difference was found both at three months and six months compared to pre-treatment levels ($p = 0.001$ and $p = 0.002$ respectively).

In the placebo treated group a similar rise in dihydrotestosterone levels was noted. Again there was a statistical difference at three months and six months as compared to pre-treatment levels ($p = 0.0005$ and 0.003 respectively). These results are expressed in histogram form in Figure 7.4.

Mean levels (± S.D.) of Dihydrotestosterone (nmol/l) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (nmol/l)	2.9	3.7	4.2
± S.D.	1.2	0.8	1.7
Mean of Placebo Group (n = 24) (nmol/l)	2.6	3.6	4.2
± S.D.	1.1	1.1	2.2

Significant changes (p =) in Dihydrotestosterone after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.001	0.002	0.24
Placebo Group (n = 24)	0.0005	0.003	0.27

Table 7.19

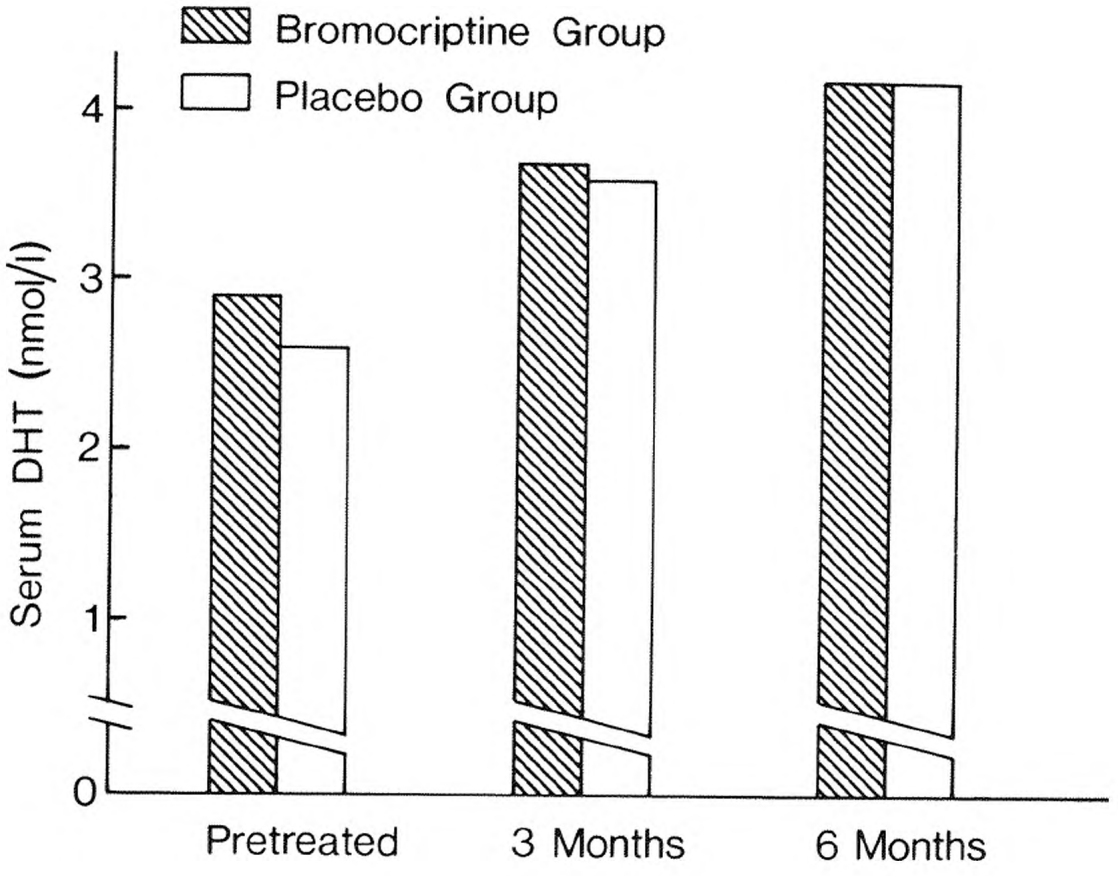


Figure 7.4 : Serum DHT (nmol/l) after treatment with Bromocriptine or Placebo

SEMINAL HORMONAL ESTIMATIONS

Seminal Prolactin (mIU/l):

With reference to Table 7.20in the Bromocriptine treated group there was a significant reduction in seminal prolactin levels after three months' treatment to a mean level of 700.9 mIU/l ($p = 0.040$). However at six months the mean seminal levels had returned to 737.5 mIU/l although this rise was not significant ($p = 0.46$). There was also no significant difference between the pre-treatment levels and the levels at six months ($p = 0.19$). In the placebo treated group no significant differences were found in either three months or six months when compared to the pre-treatment levels ($p = 0.96$ and 0.62 respectively). These results are expressed in histogram form (see Figure 7.5).

Mean levels (± S.D.) of Seminal Prolactin (mIU/1) before and after treatment with Bromocriptine or Placebo.

	PRETREATED (a & b)	TREATED 3 months	TREATED 6 months
Mean of Bromocriptine Group (n = 26) (mIU/1)	797.7	700.9	737.5
± S.D.	199.0	180.6	180.6
Mean of Placebo Group (n = 24) (mIU/1)	861.0	862.7	840.5
± S.D.	179.2	175.1	144.7

Significant changes (p =) in Seminal Prolactin after treatment with Bromocriptine or Placebo.

	PRETREATED vs 3 months	PRETREATED vs 6 months	3 months vs 6 months
Bromocriptine Group (n = 26)	0.040	0.19	0.46
Placebo Group (n = 24)	0.96	0.62	0.63

Table 7.20

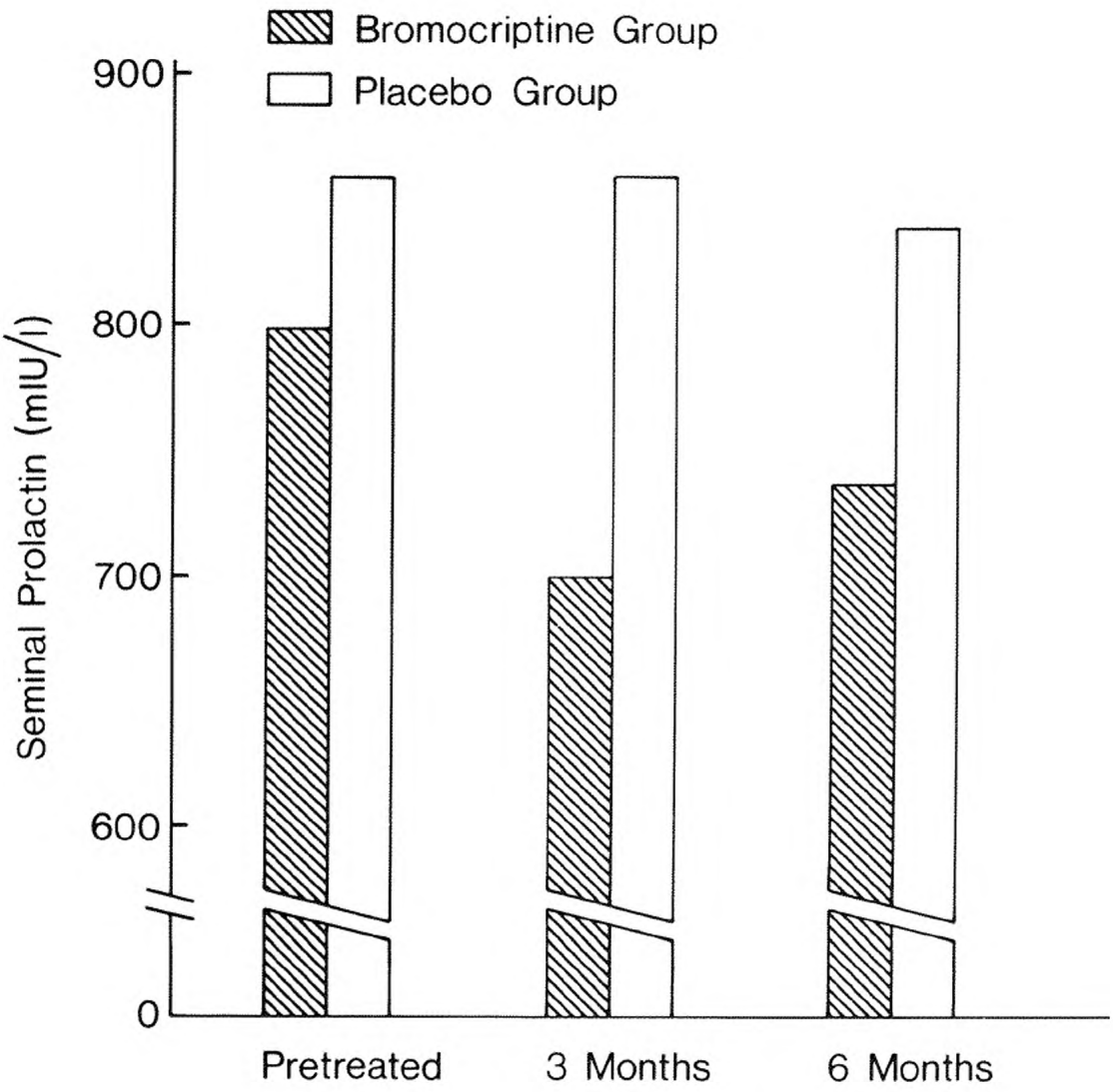


Figure 7.5: Seminal Prolactin (mIU/l) after treatment with Bromocriptine or Placebo

THE SIDE EFFECTS OF BROMOCRIPTINE

The main side effect of Bromocriptine was nausea occurring in 38% of patients initially, but persisting in only 15% of patients at 6 months. Interestingly, 4% of the placebo group also complained of nausea throughout the six months. No patient needed to cease treatment because of the nausea. Vomiting occurred in 11% of the patients falling to 7% at 6 months, whereas in the placebo group no patients initially complained of this side effect, although 4% did at 6 months. Dizziness occurred initially in 30% of the Bromocriptine treated group falling to 7% at 6 months. In the placebo group, 8% of patients reported dizziness throughout the treatment period. One patient in the Bromocriptine group complained of hallucinations and two patients complained initially of the feeling of faintness for short periods. No such side effects were reported in the placebo group.

It would appear therefore that nausea and dizziness did occur in over one third of the patients on Bromocriptine, but not severe enough to cease treatment. Slight adjustment of dosage and especially the timing of administration - i.e. with food in the evening, was all that was needed to remove the above side effects.

PREGNANCY RATE

There were seven pregnancies (14%) during the trial, of which six went to full term. Five pregnancies occurred in the placebo treated group and two in the Bromocriptine treated group. One pregnancy aborted in the placebo group at twelve weeks.

CHAPTER VIII

DISCUSSION

DISCUSSION

(a) Serum Prolactin:

The lack of statistical difference in serum prolactin levels between fertile and infertile patients conflicts with the previous work of Franks (1978), Segal (1976) and Tolis (1979). Both Franks and Segal reported a heterogeneous group of patients. No levels of FSH which would indicate the severity of the testicular disorders being studied were reported in either of these papers. Tolis (1979) in comparing the normospermic group of patients (i.e. sperm density greater than 40 million/ml) with thirteen oligospermic (i.e. sperm count less than 15 million/ml) found that although there were higher values in the infertile group, there was no significant difference ($p > 0.1$). The oligospermic group however, did have a higher FSH value as compared to the controls ($p < 0.001$) which was in contrast to this series.

Merino reporting in 1980 showed higher serum prolactin levels in idiopathic oligospermia as compared to normal men. He further sub-divided the patients on the basis of testicular biopsy results and excluded from his comparison any severe disorders of testicular function such as Klinefelter's Syndrome. However, there was still a much greater level of FSH in this group as compared to his controls.

Again, comparison of this data to that recorded in this Thesis is inappropriate, since again the FSH levels were very much higher.

The apparent effect of stress was also not apparent in this series as can be shown by the similar prolactin levels found at both visits (a) and (b). Certainly in an investigation of this order one would expect patients to be under greater stress at the initial sampling.

It therefore appears that the previous reports (Segal, 1976), Saidi, 1977) suggesting that hyperprolactinaemia results in infertility in the male, is not substantiated by the above data. It would appear that the reports of Carter (1978), Jequier (1979), Rjosk & Schill (1979) concluding that hyperprolactinaemia does not result in impaired spermatogenesis, would be the case.

CORRELATION WITH OTHER PARAMETERS:

Seminal Analysis:

As to whether serum prolactin has a direct effect on spermatogenesis is still unclear. To this extent this data agrees with Merino (1980). This is certainly underlined by the lack of correlation in the fertile group to the other parameters studied. In the infertile group the situation was somewhat different. The negative correlation of the total numbers of sperm present in the ejaculate conflicts with the further information enumerated below which shows a lowering in the serum prolactin level with Bromocriptine actually reduces the total numbers of sperm produced. No other papers have reported this parameter in comparison to serum prolactin. This is also true as far as the negative correlation found between serum prolactin and the total number of spermatozoa and the total number of normal spermatozoa found in the ejaculate. It would seem from this information that the raised serum prolactin could affect the total testicular output of spermatozoa. This depression has indeed been previously suggested by Carter (1978) although the patients incorporated in his study had serum prolactin levels very much higher due to the presence of secreting tumours of the pituitary.

The significant negative correlation of serum prolactin with seminal volume while agreeing with work in rodents (Bartke, 1977) has not been previously reported in the human. However it must be noted that this correlation is only found in the infertile group and therefore might only be reflecting a certain disorder of testicular function.

Serum Hormonal Investigations:

The correlation between serum hormonal estimations and serum prolactin in the infertile group and the lack of correlation with dihydrotestosterone conflicts with the experience of Magrini (1976). In this paper where patients had artificially induced hyperprolatinaemia, he suggested that prolactin interfered with the 5α reductase levels, thus reducing the amount of dihydrotestosterone metabolised from testosterone. The positive correlation with FSH in the infertile group might suggest a possible way that serum prolactin is affecting spermatogenesis, but whether this is a primary or secondary effect cannot be established from these data. The hypothesis put forward in the first section of this thesis suggesting a possible defect in the hypothalamic pituitary axis in infertile patients appears to be unsupported by this information.

The data presented in this series as to whether raised prolactin levels interfere with the binding of gonadotrophins to the gonad or by interference with 5α reductase activity is equivocal. To this extent there is agreement with Koskimies (1978) who also found no significant difference between the fertile and infertile patients as far as serum prolactins were concerned, but did show a negative correlation with sperm density.

(b) Seminal Prolactin:

The lack of statistical difference between the seminal prolactin levels in the fertile and infertile groups is similar to the experience of serum prolactin. However the levels of seminal prolactin appeared to be four times that found in the serum. This information conflicts with Tolis (1979) who produced evidence to show that in the normospermic man seminal prolactin levels were in the region of twice serum levels. He also reported that in the oligospermic males this concentration did not occur. In our series there appears to be no difference between the fertile and infertile groups and no such defect in concentration was established. The results also conflict with Schoenfeld (1979) who showed lower levels of seminal prolactin in thirty oligospermic males. However, in both of these papers no mention as to fertility was made; the difference between the two groups being purely on density.

Looking at the comparison between seminal prolactin and serum prolactin, interesting results occurred in the two different groups. In the fertile patients there was no significant correlation, but in the infertile group there was a highly significant positive correlation - a fact that has not previously been reported. This correlation difference in the two groups rules out the possibility that prolactin in seminal fluid is present by simple diffusion from the serum compartment. The cause for this difference is at the present time unexplained and obviously requires further investigation. One possible explanation has been put forward by Shah in 1976 who suggested that high concentrations of prolactin in seminal plasma might impair sperm motility. It is interesting to note that in this data there was a positive correlation between the seminal

prolactin and percentage live motility ($p = 0.03$).

The finding of a four-fold increase in prolactin in seminal fluid as compared to serum agrees with the work of Sheth (1975) who also found the same gradient in fertile and infertile men. At the same time they stated that the most likely source of prolactin in human semen was from the secretion from the prostate and seminal vesicles. Nothing in the above data in this series suggests otherwise. However, like them, the mechanism which controls the secretion of prolactin in the human cannot at the present time be clearly understood.

CORRELATION WITH OTHER PARAMETERS:

Seminal Analysis:

In the fertile group seminal prolactin produced similar results as the serum prolactin. Again there was no significant correlation throughout all the paramters studied at the 5% level.

However, in the infertile group there was a significant positive correlation with percentage dead cells and percentage live motility. This tends to support the work of Sheth (1979) who showed that prolactin activated the enzyme activity as far as ATPas was concerned and by doing this influenced the energy metabolism of the spermatozoa. Otherwise the lack of correlation as far as sperm density is concerned and the total numbers of spermatozoa in the ejaculate tends to suggest that seminal prolactin is not directly involved in the regulation of spermatogenesis.

Serum Hormonal Investigations:

The fertile group showed no correlation of prolactin to any of these parameters and a significant lack of correlation to serum prolactin. However, in the infertile group, a strong correlation was found with both LH, FSH at the 1% level; taking into account the quality control of the three assays involved, this finding is still significant. This information seems to conflict with most authors. Sheth et al (1973), L 'Hermite et al (1976), Segal et al (1976), Niermann et al (1977), Rjosk & Schill (1979) who showed no correlation between serum prolactin levels and LH and FSH.

Fossati in 1979 also measured serum prolactic and serum FSH and LH but no information as to the correlation between these parameters was presented.

The strong positive correlation between seminal and serum prolactin in the infertile patients is in strict contrast to that found in the fertile group. Again Fossati (1979) found that levels of seminal prolactin were approximately four times serum levels, thus agreeing with his data, but he found no significant difference between a normospermic and oligospermic group. The data from this series tend to suggest that the infertile patient has some disorder of prolactin metabolism. Even if no direct effect of this disorder has been shown in the parameters of seminal analysis studied, there still could be some link between seminal prolactin and fertility. In this place the lack of correlation with dihydrotestosterone both in the fertile and the infertile groups seems to suggest that prolactin does not appear to interfere with the metabolism of this important androgen. Therefore at serum level this data neither conflicts with nor agrees with Magrini (1976) who showed that artificial elevation of prolactin levels can interfere in the metabolism of testosterone to dihydrotestosterone.

Rjosk & Schill (1979) showed only a weak correlation ($p = 0.05$) between serum prolactin and testosterone within the oligospermic patients and doubted its biological relevance. Other authors, Sheth (1973), L 'Hermite (1976), Segal (1976), Nierman (1977) also showed no correlation between serum prolactin and testosterone.

The Effect of Prolactin-lowering Agent (Bromocriptine) in Fifty Infertile Males:

(a) Serum Prolactin:

Bromocriptine 2.5 mg b.d. reduces serum prolactin in the male, a fact already established by many workers (Segal, 1976; Saidi, 1977). That seminal prolactin followed a similar pattern to serum has not been previously established. Since it has already been suggested that the presence of prolactin in the seminal fluid is not by mere diffusion from the serum, the actual source of seminal prolactin and the method in which Bromocriptine reduces its level requires further investigation.

That Bromocriptine reduces the total number of normal and motile spermatozoa conflicts with previous workers. It must be stressed that both the work of Sheth (1975) and Saidi (1977) suggesting that Bromocriptine increased density levels in gentlemen with hyperprolactinaemia; their groups were small, poorly selected and no comparison was made to a placebo-control group. The reduction in total numbers by Bromocriptine also conflicts with previous results in this series suggesting that mildly raised prolactin levels in the serum interfere with spermatogenesis. It may be that hyperprolactinaemia noted in these patients is of a secondary nature due to another yet unidentified factor.

Since commencement of this trial, one other group has reported the use of Bromocriptine versus placebo in

two separate groups of twenty oligospermic males. In this paper by Hovatta in 1979, although they found that their patients taking Bromocriptine had overall lower sperm counts, there were no statistical differences when compared to the placebo group. They went on to state in their discussion that the favourable effects seen in a few patients taking Bromocriptine were probably due to the placebo effect and this may also explain the rapid success reported in the anecdotal cases of previous uncontrolled studies. Again, effective suppression levels of prolactin were reported in the Bromocriptine group.

The progressive fall in seminal volume at three months appears to contradict the negative correlation found between seminal volume and serum prolactin in the overall fertile group, as already stated previously. As can be seen in the placebo group there is no difference in seminal volume levels in either three or six months. Although it appears in the overall infertile group that higher prolactin levels are associated with lower seminal volume, the actual reduction in serum prolactin by Bromocriptine appears not to increase seminal volume. It is certainly interesting to note that the pattern of the fall at three months and return to normal levels at six months, is similar to that found in serum prolactin measurements. Hovatta (1979) also found no change in the seminal volume between Bromocriptine and placebo groups and again the data appear to be in agreement.

Serum Hormonal Measurements:

Bromocriptine does not appear to have an effect on serum 17β oestradiol, LH or FSH, either directly or by lowering the serum prolactin level. The lack of correlation with 17B oestradiol is not surprising, but the lack of effect on the gonadotrophins when serum prolactin is lowered seemed to conflict with the evidence already presented, suggesting that serum prolactin interfered with the action of these hormones at the gonadal level. However the fact that very few of these patients actually presented with raised gonadotrophins prior to treatment probably explains this conflict. It is interesting to note that both groups showed a significant reduction of luteinizing hormone at three months and also a significant increase at six months. This may be attributed to the confidence brought about by treatment, and might paritally explain the well known placebo effect on spermatogenesis. It is tempting to speculate that the return to pre-treated levels at six months may be due to re-activation of anxiety brought about by the knowledge that treatment was about to cease, although there is no data in this series to support such a theory.

The significant rise of dihydrotestosterone in both groups at three and six months was clearly not due to the effect of Bromocriptine. Like luteinizing hormone, this rise may be the effect of treatment by itself and again may also explain the placebo effect known to occur with most drug regimes used in male infertility. It again underlines the importance of evaluating any such regimes with a controlled placebo group. Hovatta (1979)

also noticed the steady increase in levels of testosterone and dihydrotestosterone in both the Bromocriptine and placebo groups. On the other hand Magrini (1976) observed increasing plasma dihydrotestosterone levels when serum prolactin was suppressed with Bromocriptine. However, no placebo group was incorporated for comparison. This data therefore appears to agree with Hovatta (1979) who concluded that Bromocriptine neither affects sperm count nor the major androgen levels in oligospermic infertile men.

Finally, it is interesting to note the difference in the performance of LH and dihydrotestosterone in both Bromocriptine treated and placebo treated patients. This apparent difference in performance of these apparently two closely connected parameters, probably underlines again the danger of using serum dihydrotestosterone as a marker of testicular function, since as already stated, the majority of the steroid is produced away from the testis.

Seminal Prolactin

The pattern in seminal prolactin in both the Bromocriptine treated group and the placebo treated group appears to follow that of the serum prolactin, especially in regard to a significant decrease in levels at three months in the Bromocriptine treated group. Again like serum prolactin there is an increase in levels - be it not significant - at the six months level. Whether this is due to an increasing lack of motivation as the patient sees treatment coming to an end without succes, or whether it is due to an increase in anxiety levels, is not known. What can be clearly stated is that seminal prolactin is suppressed with Bromocriptine therapy. This suppression had no effect on either sperm motility or velocity and therefore does not support the hypothesis put forward by Shah (1976) suggesting a connection.

PREGNANCY RATE

Within the context of this data Bromocriptine appears not to have a beneficial effect on fertility. The overall pregnancy rate of 14% is the accepted placebo level in a trial of this nature.

There was no significant difference in any of the parameters studied between these fertile patients and the remaining infertile ones. Considering the numbers in the fertile group, this is not at all unexpected. Since this is the first trial of this nature there is no available data for comparison.

CONCLUSIONS:

There was no statistical difference between either serum or seminal prolactin when comparing fertile or infertile patients. In infertile patients FSH levels correlate with prolactin at both serum and seminal levels. Serum LH also correlates with seminal prolactin. Fertile patients showed no such correlation suggesting that in infertile patients prolactin interferes in some way with the action of gonadotrophins on the gonad.

Prolactin does not appear to interfere with the androgen production in either the fertile or infertile patients.

Seminal prolactin levels are four times serum levels in both fertile and infertile patients. Correlations between seminal and serum prolactin levels are only found in infertile patients.

Bromocriptine is an effective prolactin-lowering agent at both serum and seminal levels.

Bromocriptine has a deleterious effect on sperm production and no apparent effect on androgen metabolism.

Finally it is concluded on the basis of the above data mildly raised serum prolactin appears not to be an aetiological factor in male subfertility. Bromocriptine, although being an effective prolactin lowering agent, does not appear to offer a new treatment for male subfertility.

APPENDIX A:

DETAILED RESULTS PART I

Sperm Velocity (μm/sec) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	37.95	41.07
02	34.41	25.68
03	29.92	33.79
04	27.88	34.99
05	25.36	27.50
06	25.24	26.36
07	24.77	31.78
08	24.73	25.25
09	32.54	33.37
10	29.11	22.32
11	31.17	29.14
12	23.34	27.71
13	33.40	27.58
14	24.00	27.43
15	31.83	26.29
16	28.32	30.76
17	24.85	18.45
18	40.45	31.68
19	27.80	27.11
20	33.87	26.31
21	38.34	30.59
22	26.11	26.53
23	27.37	20.69
24	18.39	21.66
25	26.62	26.53
26	30.69	38.67
27	22.44	18.86
28	25.58	19.57
29	22.84	22.14
30	28.69	28.18
31	21.48	20.52
32	25.91	23.83
33	20.16	19.12
34	28.43	26.00
35	33.15	27.50
36	32.94	31.49
37	30.38	31.57
38	29.68	27.34
39	30.14	28.09
40	23.42	25.89
41	29.82	31.84
42	32.80	38.21
43	25.74	24.96
44	43.27	44.92
45	32.68	40.12
46	24.94	33.37
47	21.91	28.00
48	25.83	24.16
49	22.80	25.71
50	26.99	22.58

INFERTILE (50)

NO.	(a)	(b)
51	42.17	48.09
52	37.64	37.56
53	42.91	33.75
54	35.10	34.25
55	34.31	35.74
56	38.76	39.55
57	34.94	34.83
58	32.09	29.57
59	36.50	36.97
60	34.00	38.17
61	31.63	37.00
62	32.53	37.19
63	32.36	35.69
64	32.75	35.62
65	35.36	42.46
66	34.82	34.61
67	34.47	34.66
68	35.19	38.097
69	36.16	38.44
70	37.63	35.78

FERTILE (20)

	INF.	FERT.
MEAN	28.23	36.23
SD	5.52	3.42

Table 9.1

NO.	(a)	(b)
01	07	09
02	24	10
03	07	06
04	18	13
05	10	14
06	12	19
07	16	15
08	23	18
09	15	13
10	16	14
11	33	27
12	18	23
13	07	09
14	07	12
15	16	20
16	12	12
17	20	16
18	15	18
19	16	25
20	10	20
21	14	08
22	06	06
23	18	10
24	30	17
25	19	18
26	06	18
27	12	16
28	38	28
29	21	20
30	10	15
31	10	07
32	24	24
33	24	30
34	09	10
35	22	20
36	08	08
37	23	25
38	18	09
39	21	26
40	32	32
41	21	22
42	30	10
43	22	17
44	19	21
45	12	09
46	28	14
47	16	14
48	12	11
49	08	24
50	17	17

INFERTILE (50)

NO.	(a)	(b)
51	18	13
52	27	20
53	30	18
54	15	15
55	08	15
56	07	12
57	15	16
58	16	11
59	07	08
60	28	21
61	15	21
62	16	13
63	20	11
64	12	14
65	12	14
66	22	27
67	06	06
68	26	15
69	09	13
70	07	12

FERTILE (20)

	INF.	FERT.
MEAN	16.7	15.2
SD	7.1	6.3

Table 9.2

NO.	(a)	(b)
01	76	78
02	68	57
03	73	82
04	62	46
05	78	79
06	60	65
07	65	72
08	60	69
09	73	67
10	61	62
11	53	59
12	66	61
13	50	58
14	47	58
15	71	63
16	66	69
17	55	66
18	60	71
19	52	63
20	61	50
21	65	71
22	70	70
23	73	60
24	40	48
25	54	53
26	62	63
27	57	62
28	39	44
29	61	48
30	54	66
31	57	46
32	63	64
33	51	45
34	69	57
35	67	73
36	67	67
37	66	60
38	63	62
39	54	60
40	47	41
41	60	79
42	70	68
43	52	66
44	80	81
45	71	67
46	54	74
47	72	58
48	40	39
49	62	58
50	52	52

INFERTILE (50)

NO.	(a)	(b)
51	74	82
52	49	59
53	55	57
54	74	64
55	72	66
56	73	66
57	65	49
58	54	64
59	71	59
60	57	61
61	63	48
62	51	51
63	76	71
64	73	71
65	72	70
66	53	39
67	72	72
68	47	59
69	52	67
70	72	61

FERTILE (20)

	INF.	FERT.
MEAN	61.4	62.7
SD	10.09	9.9

Table 9.3

Percentage Live Motility (%) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	82	85
02	89	63
03	78	87
04	75	53
05	87	92
06	69	81
07	77	85
08	78	84
09	86	77
10	73	72
11	74	80
12	81	79
13	54	64
14	50	66
15	84	78
16	75	78
17	69	78
18	70	86
19	62	85
20	67	63
21	76	77
22	75	75
23	87	67
24	57	57
25	67	68
26	66	77
27	64	74
28	62	62
29	77	60
30	59	78
31	63	49
32	82	85
33	67	65
34	76	63
35	85	91
36	73	73
37	86	80
38	77	68
39	69	81
40	69	61
41	77	99
42	99	75
43	67	79
44	99	99
45	81	73
46	74	86
47	86	68
48	46	44
49	67	76
50	63	63

INFERTILE (50)

NO.	(a)	(b)
51	90	94
52	66	74
53	79	69
54	87	75
55	79	77
56	78	75
57	76	58
58	64	72
59	76	64
60	80	77
61	74	60
62	61	59
63	95	80
64	83	83
65	82	82
66	68	54
67	77	77
68	63	69
69	57	77
70	78	70

FERTILE (20)

	INF.	FERT.
MEAN	73.89	73.9
SD	11.4	9.8

Table 9.4

Percentage of Abnormal Forms (%) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	18	20
02	11	30
03	23	23
04	12	28
05	70	45
06	38	50
07	45	48
08	35	39
09	20	20
10	46	35
11	33	44
12	23	25
13	14	27
14	66	92
15	36	33
16	27	25
17	40	45
18	49	33
19	26	25
20	23	33
21	21	27
22	18	17
23	26	33
24	70	50
25	70	69
26	48	25
27	30	27
28	25	25
29	25	30
30	21	20
31	21	35
32	40	37
33	60	70
34	70	45
35	21	20
36	38	38
37	35	30
38	33	30
39	35	35
40	38	55
41	85	65
42	12	25
43	66	60
44	35	54
45	21	45
46	50	50
47	70	57
48	35	35
49	73	80
50	80	75

INFERTILE (50)

NO.	(a)	(b)
51	27	27
52	64	42
53	55	45
54	38	55
55	34	54
56	20	32
57	50	45
58	55	50
59	30	50
60	45	50
61	40	65
62	50	35
63	25	30
64	55	50
65	33	40
66	52	40
67	33	40
68	60	55
69	50	37
70	30	40

FERTILE (20)

	INF.	FERT.
MEAN	39.1	43.2
SD	18.6	11.2

Table 9.5

Density (x10^6/ml) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	022	021
02	019	012
03	030	039
04	056	105
05	004	004
06	035	047
07	034	028
08	021	022
09	042	081
10	110	096
11	012	013
12	048	063
13	095	115
14	006	007
15	021	016
16	068	062
17	005	003
18	029	028
19	008	037
20	018	017
21	033	013
22	105	137
23	005	016
24	015	016
25	024	023
26	040	125
27	013	016
28	068	077
29	046	036
30	071	045
31	071	093
32	008	005
33	011	016
34	015	021
35	041	045
36	026	026
37	022	029
38	032	033
39	016	022
40	071	079
41	048	014
42	139	098
43	006	007
44	053	066
45	068	054
46	020	036
47	004	009
48	076	134
49	006	005
50	010	006

INFERTILE (50)

NO.	(a)	(b)
51	053	087
52	058	062
53	101	103
54	052	056
55	100	092
56	113	055
57	044	052
58	035	044
59	091	075
60	178	189
61	085	099
62	039	036
63	155	125
64	038	032
65	087	085
66	086	094
67	068	078
68	034	032
69	045	040
70	103	202

FERTILE (20)

	INF.	FERT.
MEAN	39×10^6	80×10^6
SD	34×10^6	42×10^6

Table 9.6

Total Number of Spermatozoa (millions) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	66	57
02	76	58
03	118	146
04	140	683
05	9	9
06	63	131
07	105	39
08	69	110
09	88	146
10	253	220
11	30	31
12	238	315
13	285	460
14	7	15
15	59	51
16	265	217
17	18	18
18	106	62
19	24	73
20	65	44
21	231	60
22	263	274
23	24	48
24	45	77
25	72	64

NO.	(a)	(b)
26	44	125
27	26	19
28	240	400
29	83	49
30	390	121
31	390	502
32	32	22
33	64	86
34	26	86
35	131	162
36	109	109
37	97	110
38	186	125
39	56	110
40	184	190
41	173	50
42	181	167
43	16	21
44	186	178
45	177	86
46	52	144
47	13	31
48	228	456
49	11	6
50	40	18

INFERTILE (50)

NO.	(a)	(b)
51	106	156
52	139	118
53	191	154
54	312	352
55	160	156
56	395	176
57	132	120
58	105	110
59	164	120
60	641	378
61	459	594
62	176	144
63	527	450
64	99	77
65	200	221
66	189	207
67	156	94
68	136	93
69	162	140
70	257	646

FERTILE (20)

	INF.	FERT.
MEAN	127	230
SD	124	158

Table 9.7

Total Number of Live Spermatozoa (millions) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	61	52
02	58	52
03	110	137
04	114	594
05	8	8
06	55	107
07	88	33
08	53	90
09	75	127
10	212	189
11	20	23
12	195	243
13	265	418
14	7	14
15	49	41
16	233	191
17	14	15
18	90	51
19	20	55
20	58	35
21	199	55
22	247	257
23	20	43
24	31	64
25	58	52
26	41	103
27	23	16
28	210	288
29	65	37
30	351	104
31	351	467
32	24	17
33	48	60
34	23	77
35	102	129
36	100	100
37	74	83
38	152	114
39	44	81
40	125	129
41	137	39
42	126	150
43	12	17
44	150	140
45	155	79
46	37	124
47	11	26
48	201	405
49	10	4
50	33	15

INFERTILE (50)

NO.	(a)	(b)
51	87	136
52	101	94
53	134	126
54	265	300
55	147	133
56	368	154
57	112	100
58	88	98
59	152	110
60	461	298
61	390	469
62	147	125
63	421	400
64	87	66
65	176	190
66	147	151
67	147	88
68	101	79
69	147	121
70	239	568

FERTILE (20)

	INF.	FERT.
MEAN	129	193
SD	108	106

Table 9.8

Total Number of Motile Spermatozoa (millions) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	50	44
02	51	33
03	86	120
04	86	316
05	7	7
06	38	86
07	68	28
08	41	75
09	64	98
10	154	136
11	16	18
12	157	193
13	143	268
14	3	9
15	41	32
16	176	149
17	10	12
18	63	44
19	13	46
20	39	22
21	150	42
22	185	192
23	17	29
24	18	36
25	39	44
26	27	79
27	15	12
28	132	178
29	50	23
30	209	80
31	224	230
32	20	14
33	32	39
34	18	49
35	87	118
36	73	73
37	64	66
38	117	78
39	30	66
40	87	79
41	105	40
42	126	113
43	8	13
44	148	144
45	125	58
46	28	107
47	10	18
48	92	177
49	7	3
50	21	9

INFERTILE (50)

NO.	(a)	(b)
51	78	128
52	67	70
53	105	88
54	231	225
55	116	103
56	287	116
57	86	58
58	56	70
59	116	71
60	367	229
61	286	282
62	90	74
63	402	320
64	72	55
65	144	155
66	101	82
67	113	68
68	63	55
69	84	93
70	186	397

FERTILE (20)

	INF.	FERT.
MEAN	75	144
SD	65	101

Table 9.9

and Fertile (20) Patients:

NO.	(a)	(b)
01	54	45
02	68	40
03	91	113
04	123	491
05	3	5
06	39	65
07	58	20
08	45	67
09	71	116
10	136	143
11	20	18
12	183	236
13	245	335
14	2	1
15	38	34
16	193	162
17	11	10
18	54	41
19	18	55
20	50	30
21	182	43
22	215	227
23	80	32
24	14	38
25	22	20
26	22	94
27	18	14
28	255	300
29	62	33
30	308	97
31	308	326
32	19	14
33	25	26
34	9	47
35	103	130
36	67	67
37	62	77
38	124	88
39	36	72
40	114	85
41	26	18
42	159	125
43	5	8
44	121	82
45	139	48
46	26	72
47	4	13
48	148	296
49	3	1
50	8	5

INFERTILE (50)

NO.	(a)	(b)
51	77	114
52	50	68
53	86	84
54	193	158
55	106	72
56	314	120
57	66	66
58	47	55
59	114	60
60	352	189
61	275	208
62	88	93
63	395	315
64	44	38
65	134	133
66	90	124
67	104	56
68	54	42
69	81	88
70	180	388

FERTILE (20)

	INF.	FERT.
MEAN	86	133
SD	93	99

Table 9.10

NO.	(a)	(b)
01	3.0	2.7
02	4.0	5.0
03	6.0	3.7
04	2.5	6.5
05	2.5	2.3
06	1.8	2.8
07	3.1	1.4
08	3.3	5.0
09	2.1	1.8
10	2.3	2.3
11	2.6	2.5
12	5.0	5.0
13	3.0	4.0
14	1.3	2.2
15	2.8	3.2
16	3.9	3.5
17	4.0	6.0
18	3.6	2.2
19	3.0	2.0
20	3.7	2.6
21	7.0	4.6
22	2.5	2.0
23	4.8	3.0
24	3.0	4.8
25	3.0	2.8
26	1.1	1.0
27	2.0	1.2
28	5.0	5.2
29	1.8	1.3
30	5.5	2.7
31	5.5	5.4
32	4.0	4.4
33	5.8	5.4
34	1.7	4.2
35	3.2	3.6
36	4.2	4.2
37	4.4	3.8
38	5.8	3.8
39	3.5	5.0
40	2.6	2.4
41	3.6	3.6
42	1.3	1.7
43	2.6	3.2
44	3.5	2.7
45	2.6	1.6
46	2.6	4.0
47	3.8	3.6
48	3.0	3.4
49	1.8	1.3
50	4.0	3.0

INFERTILE (50)

NO.	(a)	(b)
51	2.0	1.8
52	2.4	1.9
53	1.9	1.5
54	6.0	6.3
55	1.6	1.7
56	3.5	3.2
57	3.0	2.3
58	3.0	2.5
59	1.8	1.6
60	3.6	2.0
61	5.4	6.0
62	4.5	4.0
63	3.4	3.6
64	2.6	2.4
65	2.3	2.6
66	2.2	2.2
67	2.3	1.2
68	4.0	2.9
69	3.6	3.5
70	2.5	3.2

FERTILE (20)

	INF.	FERT.
MEAN	3.3	2.9
SD	1.3	1.2

Table 9.11

Patients:

NO.	(a)	(b)
01	260	240
02	210	190
03	180	180
04	190	190
05	200	210
06	180	280
07	210	240
08	180	200
09	240	170
10	280	230
11	210	150
12	160	200
13	160	160
14	230	220
15	230	210
16	160	115
17	140	120
18	110	115
19	160	180
20	150	110
21	210	190
22	180	115
23	250	210
24	170	370
25	250	190
26	240	75
27	135	45
28	10	25
29	40	30
30	100	125
31	70	125
32	110	150
33	75	180
34	210	100
35	210	145
36	140	195
37	370	180
38	220	160
39	130	35
40	270	230
41	260	185
42	100	150
43	205	250
44	120	110
45	120	100
46	280	160
47	140	120
48	170	120
49	100	110
50	170	180

INFERTILE (50)

NO.	(a)	(b)
51	240	110
52	140	145
53	225	220
54	210	205
55	180	30
56	110	105
57	130	105
58	210	90
59	95	90
60	85	50
61	100	115
62	240	20
63	90	45
64	50	20
65	230	140
66	112	220
67	260	250
68	200	140
69	200	140
70	110	110

FERTILE (20)

	INF.	FERT.
MEAN	168.8	139.3
SD	68.5	68.8

Table 9.12

Serum Prolactin (mIU/l) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	156	105
02	150	144
03	144	143
04	170	131
05	395	344
06	267	241
07	104	169
08	134	117
09	193	167
10	215	152
11	170	209
12	130	167
13	325	158
14	192	311
15	182	177
16	185	148
17	188	166
18	134	167
19	290	215
20	510	305
21	215	200
22	382	158
23	106	143
24	291	338
25	171	144
26	479	363
27	128	102
28	210	206
29	268	308
30	180	263
31	108	116
32	250	216
33	273	218
34	157	143
35	163	199
36	192	238
37	301	209
38	337	181
39	255	188
40	224	269
41	362	230
42	158	108
43	216	156
44	360	206
45	240	285
46	260	372
47	279	341
48	188	188
49	151	206
50	249	302

INFERTILE (50)

NO.	(a)	(b)
51	264	246
52	166	106
53	194	148
54	244	250
55	286	204
56	203	282
57	156	165
58	245	270
59	182	361
60	367	320
61	142	156
62	181	157
63	200	235
64	138	168
65	197	145
66	210	102
67	112	119
68	439	214
69	510	457
70	130	125

FERTILE (20)

	INF.	FERT.
MEAN	220.2	219.9
SD	84.8	97.5

Table 9.13

NO.	(a)	(b)
01	4.1	3.4
02	6.4	9.0
03	2.9	4.1
04	4.1	4.8
05	12.0	6.8
06	7.8	7.8
07	5.4	4.4
08	2.7	3.8
09	6.0	6.2
10	4.8	7.7
11	4.0	8.0
12	4.4	4.0
13	5.8	5.6
14	3.8	6.1
15	2.9	3.3
16	3.5	4.0
17	3.6	4.7
18	5.4	6.8
19	7.2	7.4
20	1.6	9.2
21	5.8	3.4
22	5.7	5.3
23	3.6	2.5
24	4.6	3.3
25	7.8	5.9
26	4.3	3.4
27	4.2	5.4
28	3.4	3.8
29	5.8	4.8
30	3.9	3.8
31	3.0	3.7
32	11.0	7.2
33	3.5	8.1
34	5.0	5.8
35	4.5	4.4
36	5.0	5.8
37	3.6	5.1
38	7.2	8.0
39	5.5	5.0
40	4.7	4.7
41	2.7	3.4
42	3.5	4.1
43	3.1	3.3
44	4.0	3.2
45	3.7	4.8
46	2.7	3.6
47	3.4	5.5
48	3.5	3.7
49	6.0	10.1
50	3.5	5.2

INFERTILE (50)

NO.	(a)	(b)
51	6.2	4.8
52	5.4	6.9
53	5.4	5.3
54	5.7	6.3
55	5.6	5.5
56	5.6	4.8
57	4.2	4.8
58	5.4	7.7
59	3.0	3.6
60	2.7	2.1
61	5.7	4.0
62	8.0	5.1
63	5.4	3.3
64	5.6	4.0
65	4.8	4.9
66	3.9	5.6
67	2.8	3.3
68	9.2	11.7
69	6.2	6.4
70	3.4	3.5

FERTILE (20)

	INF.	FERT.
MEAN	5.0	5.1
SD	1.9	1.8

Table 9.14

NO.	(a)	(b)
01	4.0	5.0
02	6.0	7.0
03	1.2	11.6
04	1.0	5.6
05	9.2	8.9
06	2.7	3.3
07	0.9	0.7
08	1.6	2.0
09	2.1	4.4
10	1.6	4.5
11	3.0	6.1
12	1.1	1.0
13	3.2	6.6
14	4.8	6.7
15	2.8	2.8
16	1.2	1.7
17	4.8	5.2
18	3.9	4.6
19	6.8	9.3
20	2.3	2.1
21	2.5	1.7
22	1.2	2.4
23	0.8	0.5
24	0.8	0.6
25	3.9	4.9
26	6.2	6.3
27	0.8	0.7
28	1.0	2.1
29	3.9	4.7
30	2.5	2.5
31	0.8	0.6
32	11.0	12.4
33	2.1	4.8
34	1.4	2.9
35	1.7	1.8
36	2.9	6.0
37	0.8	1.2
38	3.1	3.5
39	1.8	6.0
40	1.9	2.4
41	2.6	2.9
42	3.3	11.2
43	0.8	1.0
44	2.1	3.3
45	1.2	1.5
46	0.8	4.6
47	3.7	7.0
48	0.8	0.9
49	4.8	5.8
50	3.7	4.9

INFERTILE (50)

NO.	(a)	(b)
51	3.9	3.0
52	2.2	2.7
53	2.3	1.4
54	4.0	3.0
55	1.6	2.0
56	11.2	10.9
57	1.3	1.0
58	3.6	3.6
59	1.8	3.6
60	0.8	0.9
61	1.4	1.4
62	5.4	3.4
63	2.3	1.8
64	4.2	2.8
65	1.1	0.6
66	1.8	1.8
67	3.3	3.0
68	3.3	3.2
69	2.1	1.9
70	1.5	1.2

FERTILE (20)

	INF.	FERT.
MEAN	3.4	2.8
SD	2.6	2.2

Table 9.15

Serum DHT (nmol/1) in Infertile (50) and Fertile (20) Patients:

NO.	(a)	(b)
01	4.4	2.6
02	2.8	3.1
03	2.1	3.3
04	3.3	3.2
05	5.2	2.8
06	6.2	2.6
07	3.8	3.0
08	2.5	2.9
09	6.1	3.8
10	3.8	4.9
11	2.8	2.5
12	2.1	2.2
13	1.8	2.5
14	3.3	3.0
15	3.3	3.6
16	2.8	1.3
17	1.2	1.2
18	1.5	2.3
19	3.2	6.1
20	4.0	2.0
21	1.1	2.2
22	1.8	1.9
23	2.7	1.7
24	1.6	4.8
25	2.6	2.2
26	1.2	1.7
27	3.5	1.9
28	1.3	1.3
29	2.7	2.3
30	2.5	3.0
31	3.3	2.9
32	2.3	2.4
33	3.0	3.4
34	1.6	3.2
35	2.3	2.4
36	2.2	1.5
37	1.3	1.5
38	1.4	2.0
39	1.5	2.2
40	2.4	2.3
41	3.2	2.3
42	1.1	2.2
43	5.4	4.6
44	3.2	2.6
45	1.3	2.2
46	4.3	4.6
47	2.6	3.3
48	3.9	2.7
49	3.3	3.7
50	3.7	4.9

INFERTILE (50)

NO.	(a)	(b)
51	4.0	4.0
52	2.9	2.4
53	3.7	2.6
54	4.7	4.4
55	2.9	2.5
56	3.2	2.7
57	3.4	3.6
58	2.7	2.9
59	1.7	2.8
60	2.0	3.9
61	1.7	2.4
62	1.4	1.5
63	4.3	4.4
64	2.6	3.2
65	4.2	2.3
66	2.8	2.7
67	3.2	3.2
68	2.5	2.4
69	2.5	2.0
70	3.6	3.3

FERTILE (20)

	INF.	FERT.
MEAN	2.7	2.9
SD	1.1	0.8

Table 9.16

NO.	(a)	(b)
01	++	++
02	++	++
03	+	+
04	+	+
05	+++	+++
06	++	++
07	++	++
08	++	++
09	++	+
10	++	++
11	+++	+++
12	++	++
13	++	++
14	++	++
15	++	++
16	++	++
17	+	+
18	++	++
19	++	++
20	++	++
21	++	++
22	+	+
23	+++	++
24	++	++
25	++	++
26	+	+
27	++	++
28	++	++
29	++	++
30	++	++
31	+++	+++
32	++	++
33	++	++
34	++	++
35	++	++
36	+++	++
37	+++	++
38	++	++
39	+	+
40	++	++
41	+++	+++
42	++	++
43	++	++
44	++	++
45	++	++
46	++	++
47	+++	+++
48	++	++
49	++	++
50	++	++

INFERTILE (50)

NO.	(a)	(b)
51	+	+
52	+	+
53	++	++
54	++	++
55	++	++
56	++	+++
57	++	++
58	++	++
59	+	++
60	+	++
61	++	+++
62	++	++
63	++	++
64	++	++
65	++	++
66	++	++
67	+	+
68	++	++
69	++	++
70	++	++

FERTILE (20)

Table 9.17

APPENDIX B:

DETAILED RESULTS PART II

Serum Prolactin (mIU/1) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	47	51
05	43	82
06	82	65
08	26	59
10	52	228
12	26	31
14	77	250
15	40	456
16	56	505
19	275	270
21	87	29
22	56	319
25	41	29

No.	3/12	6/12
27	141	195
30	183	200
32	69	107
33	257	231
34	32	37
37	66	43
38	248	362
40	53	76
45	44	32
46	39	35
48	35	35
49	35	52
50	64	76

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	73	92
03	112	172
04	152	165
07	85	145
09	265	210
11	152	260
13	202	230
17	193	255
18	186	158
20	188	270
23	80	92
24	299	446

No.	3/12	6/12
26	310	414
28	255	209
29	169	221
31	114	149
35	231	197
36	218	196
39	189	219
41	213	258
42	227	243
43	121	254
44	170	217
47	367	156

(b) PLACEBO TREATED GROUP

Table 10.1

Seminal Prolactin (mIU/l) in Infertile (50) and Fertile (20) Patients.

NO.	A	B
01	545	548
02	1100	1100
03	680	775
04	665	972
05	1355	1115
06	818	920
07	755	670
08	885	870
09	1010	1090
10	965	998
11	990	995
12	803	1190
13	835	1045
14	463	568
15	940	933
16	605	500
17	1080	665
18	685	775
19	775	770
20	865	678
21	430	420
22	730	585
23	840	250
24	960	825
25	775	940
26	945	1230
27	615	460
28	850	830
29	955	995
30	840	880
31	700	665
32	625	695
33	870	880
34	1015	865
35	940	985
36	950	810
37	760	695
38	910	845
39	565	805
40	940	985
41	840	825
42	1065	900
43	760	545
44	1000	1000
45	705	990
46	745	770
47	995	870
48	720	545
49	970	1010
50	950	870

INFERTILE (50)

NO.	A	B
51	938	890
52	1165	1305
53	795	473
54	818	955
55	900	835
56	1055	995
57	653	758
58	655	575
59	945	775
60	830	705
61	755	800
62	870	805
63	875	930
64	760	660
65	805	800
66	1140	1090
67	885	1050
68	740	705
69	1090	1050
70	795	895

FERTILE (20)

	INF.	FERT.
MEAN	828.5	863.5
SD	191.5	168.5

Table 10.2(a)

Seminal Prolactin (mIU/1) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	680	685
05	833	910
06	598	770
08	763	790
10	765	705
12	782	860
14	653	653
15	715	908
16	428	603
19	807	615
21	275	355
22	622	413
25	760	700

No.	3/12	6/12
27	685	605
30	795	775
32	445	580
33	1005	975
34	815	925
37	275	515
38	805	1210
40	860	767
45	725	745
46	765	665
48	580	775
49	915	885
50	785	735

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	990	1140
03	900	768
04	915	660
07	980	995
09	698	798
11	1045	870
13	910	620
17	825	770
18	638	613
20	625	810
23	840	750
24	780	875

No.	3/12	6/12
26	1010	795
28	900	885
29	775	950
31	680	665
35	945	870
36	875	940
39	640	704
41	1265	815
42	1075	1090
43	515	760
44	820	900
47	1010	1050

(b) PLACEBO TREATED GROUP

Table 10.2(b)

Sperm Velocity (μm/sec) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	33.89	32.96
05	27.79	35.44
06	26.21	27.87
08	29.37	19.23
10	27.75	31.85
12	26.52	28.81
14	22.75	18.45
15	27.17	23.33
16	32.80	23.07
19	32.42	27.77
21	34.57	34.44
22	32.81	26.54
25	30.89	22.44

No.	3/12	6/12
27	28.27	22.49
30	37.66	36.02
32	24.27	21.69
33	20.16	27.58
34	20.24	24.31
37	30.23	30.23
38	26.89	28.81
40	25.96	16.70
45	19.59	24.90
46	24.29	34.77
48	15.60	24.86
49	21.61	19.79
50	24.46	23.93

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	30.23	24.63
03	30.30	28.05
04	26.28	36.10
07	24.49	33.60
09	28.42	36.26
11	21.02	31.48
13	31.24	29.03
17	18.86	28.05
18	32.59	41.81
20	27.83	28.49
23	23.66	26.71
24	24.90	28.88

No.	3/12	6/12
26	35.58	36.96
28	33.22	18.58
29	24.67	31.97
31	26.34	33.20
35	21.19	29.79
36	42.95	32.12
39	22.86	20.69
41	22.93	26.49
42	34.40	29.95
43	21.97	23.97
44	39.31	25.77
47	22.16	20.26

(b) PLACEBO TREATED GROUP

Table 10.3

Percentage Dead Cells (%) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	6	6
05	9	16
06	17	9
08	2	20
10	4	14
12	8	10
14	18	18
15	13	23
16	12	14
19	13	18
21	10	6
22	9	9
25	15	13

No.	3/12	6/12
27	17	11
30	13	8
32	10	33
33	19	11
34	20	16
37	25	32
38	25	5
40	22	54
45	31	8
46	11	11
48	4	18
49	20	11
50	16	11

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	10	15
03	11	9
04	17	9
07	14	18
09	15	21
11	34	32
13	13	19
17	12	27
18	21	6
20	14	23
23	14	10
24	14	13

No.	3/12	6/12
26	16	10
28	22	52
29	11	12
31	4	9
35	17	19
36	14	14
39	21	28
41	11	37
42	14	12
43	16	16
44	21	17
47	16	15

(b) PLACEBO TREATED GROUP

Table 10.4

Percentage Motility (%) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	77	77
05	66	79
06	64	65
08	68	47
10	63	62
12	66	75
14	56	54
15	46	58
16	68	61
19	75	50
21	80	69
22	75	61
25	67	39

No.	3/12	6/12
27	60	53
30	65	67
32	76	56
33	51	48
34	60	57
37	51	44
38	57	60
40	50	23
45	31	58
46	50	64
48	34	45
49	59	47
50	62	51

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	72	72
03	69	64
04	67	51
07	56	55
09	64	51
11	52	71
13	61	61
17	50	55
18	52	74
20	78	63
23	73	74
24	51	50

No.	3/12	6/12
26	64	58
28	50	34
29	61	68
31	68	64
35	63	76
36	80	64
39	54	60
41	54	56
42	68	63
43	51	48
44	76	66
47	51	60

(b) PLACEBO TREATED GROUP

Table 10.5

Percentage Live Motility (%) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months

No.	3/12	6/12
01	82	82
05	73	94
06	77	72
08	69	59
10	65	72
12	71	84
14	68	66
15	53	75
16	78	71
19	86	61
21	88	73
22	83	67
25	79	45
27	72	60
30	75	73
32	85	84
33	63	54
34	75	68
37	66	64
38	76	64
40	64	50
45	45	63
46	56	72
48	35	55
49	74	53
50	73	57

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	83	85
03	78	70
04	81	56
07	65	68
09	75	64
11	80	99
13	71	75
17	57	76
18	66	79
20	91	82
23	85	82
24	59	57
26	77	64
28	65	71
29	68	77
31	71	71
35	75	94
36	93	74
39	69	84
41	61	89
42	79	72
43	61	57
44	96	79
47	60	70

(b) PLACEBO TREATED GROUP

Table 10.6

Percentage Abnormal Forms (%) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	23	35
05	50	63
06	50	40
08	33	58
10	38	50
12	30	43
14	67	90
15	40	70
16	30	45
19	33	50
21	28	43
22	34	32
25	65	64

No.	3/12	6/12
27	50	75
30	50	53
32	30	55
33	80	77
34	85	80
37	40	70
38	53	50
40	50	73
45	70	35
46	60	65
48	45	40
49	70	72
50	68	80

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	21	33
03	25	40
04	30	35
07	65	60
09	40	35
11	57	60
13	50	40
17	40	50
18	25	45
20	35	57
23	43	60
24	55	60

No.	3/12	6/12
26	50	50
28	50	40
29	18	37
31	30	50
35	46	50
36	60	50
39	50	63
41	85	90
42	20	38
43	70	75
44	50	55
47	62	65

(b) PLACEBO TREATED GROUP

Table 10.7

Sperm Density (millions/ml) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	17	14
05	6	14
06	95	51
08	17	23
10	10	55
12	60	46
14	13	9
15	15	8
16	58	10
19	24	28
21	32	30
22	79	88
25	28	18

No.	3/12	6/12
27	29	46
30	83	89
32	5	3
33	10	12
34	15	18
37	25	28
38	29	16
40	113	83
45	46	3
46	26	23
48	167	60
49	8	8
50	8	7

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	21	6
03	26	20
04	46	128
07	19	3
09	75	41
11	19	6
13	64	96
17	2	2
18	28	28
20	17	28
23	3	5
24	18	14

No.	3/12	6/12
26	36	27
28	81	59
29	57	47
31	82	75
35	45	28
36	16	26
39	12	18
41	25	27
42	76	82
43	5	8
44	22	45
47	6	3

(b) PLACEBO TREATED GROUP

Table 10.8

Total Number of Spermatozoa (millions) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	54	21
05	11	32
06	152	107
08	33	138
10	9	72
	240	147
14	27	18
15	30	21
16	168	27
19	54	56
21	150	156
22	126	281
25	101	76

No.	3/12	6/12
27	75	96
30	157	267
32	11	16
33	29	60
34	44	58
37	11	157
38	122	64
40	294	166
45	115	8
46	156	138
48	467	102
49	7	9
50	18	14

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	78	20
03	104	100
04	313	461
07	63	11
09	98	103
11	38	12
13	121	345
17	10	10
18	48	50
20	41	129
23	11	14
24	63	59

No.	3/12	6/12
26	36	27
28	470	649
29	68	66
31	246	315
35	158	67
36	67	88
39	58	72
41	105	132
42	106	139
43	9	14
44	55	126
47	19	9

(b) PLACEBO TREATED GROUP

Table 10.9

Total Number of Live Spermatozoa (millions) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	51	20
05	10	27
06	126	97
08	32	110
10	8	61
12	221	132
14	22	15
15	26	16
16	148	23
19	47	46
21	135	147
22	115	256
25	86	66

No.	3/12	6/12
27	63	85
30	137	245
32	10	11
33	23	53
34	35	49
37	6	106
38	91	61
40	229	76
45	79	7
46	139	122
48	449	84
49	5	8
50	15	12

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	70	17
03	93	91
04	260	419
07	54	9
09	83	81
11	25	8
13	105	280
17	9	7
18	38	47
20	35	99
23	10	12
24	54	51

No.	3/12	6/12
26	30	24
28	366	312
29	61	58
31	236	287
35	131	54
36	58	76
39	45	52
41	94	83
42	92	122
43	8	12
44	43	104
47	16	8

(b) PLACEBO TREATED GROUP

Table 10.10

Total Number of Motile Spermatozoa (millions) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12	No.	3/12	6/12
01	42	16	27	45	51
05	7	25	30	103	179
06	98	70	32	8	9
08	22	65	33	14	29
10	6	44	34	26	33
12	158	111	37	7	69
14	15	9	38	70	39
15	14	12	40	147	38
16	115	16	45	36	4
19	40	28	46	79	88
21	120	107	48	159	46
22	95	173	49	4	4
25	68	30	50	11	7

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12	No.	3/12	6/12
02	56	15	26	23	15
03	72	64	28	237	221
04	210	234	29	42	44
07	35	6	31	168	203
09	62	52	35	99	51
11	20	8	36	54	56
13	75	210	39	31	43
17	5	6	41	57	75
18	25	37	42	72	88
20	32	81	43	5	7
23	8	10	44	42	83
24	32	29	47	9	5

(b) PLACEBO TREATED GROUP

Table 10.11

Total Number of Normal Spermatozoa (millions) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	42	14
05	6	12
06	76	64
08	22	58
10	6	36
12	168	84
14	9	2
15	18	6
16	118	15
19	36	28
21	108	89
22	83	191
25	35	27

No.	3/12	6/12
27	38	24
30	79	125
32	8	7
33	6	14
34	7	12
37	7	47
38	57	32
40	147	45
45	35	5
46	62	48
48	257	61
49	2	3
50	6	3

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	61	14
03	78	60
04	219	299
07	22	4
09	59	67
11	16	5
13	61	207
17	6	5
18	36	28
20	26	55
23	6	5
24	28	24

No.	3/12	6/12
26	18	14
28	235	389
29	56	41
31	172	158
35	85	37
36	27	44
39	29	27
41	16	13
42	85	86
43	3	4
44	27	57
47	7	3

(b) PLACEBO TREATED GROUP

Table 10.12

Seminal Volume (ml) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	3.2	1.5
05	2.0	2.3
06	1.6	2.1
08	2.0	6.0
10	0.9	1.3
12	4.0	3.2
14	2.0	2.0
15	2.0	2.6
16	2.9	2.7
19	2.2	2.0
21	4.7	5.2
22	1.6	3.2
25	3.6	4.2

No.	3/12	6/12
27	2.6	2.1
30	1.9	3.0
32	2.2	5.4
33	3.0	5.0
34	3.0	3.3
37	4.5	5.6
38	4.2	4.0
40	2.6	2.0
45	2.5	2.5
46	6.0	6.0
48	2.8	1.7
49	0.8	1.2
50	2.2	2.0

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	3.7	3.4
03	4.0	5.0
04	6.8	3.6
07	3.3	3.7
09	1.3	2.5
11	2.0.	2.0
13	1.9	3.6
17	5.0	4.6
18	1.7	1.8
20	2.4	4.6
23	3.8	3.0
24	3.5	4.2

No.	3/12	6/12
26	1.0	1.0
28	5.8	9.9
29	1.2	1.4
31	3.0	4.2
35	3.5	2.4
36	4.2	3.4
39	5.0	4.0
41	4.3	4.9
42	1.4	1.7
43	2.1	1.8
44	2.5	2.8
47	3.1	3.0

(b) PLACEBO TREATED GROUP

Table 10.13

Serum 17β Oestradiol (pmol/l) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12	No.	3/12	6/12
01	185	170	27	60	20
05	210	255	30	60	25
06	290	180	32	170	130
08	140	160	33	210	200
10	220	280	34	145	580
12	270	230	37	180	160
14	250	210	38	160	250
15	210	250	40	130	190
16	115	40	45	110	110
19	130	130	46	170	160
21	200	215	48	160	80
22	130	140	49	110	100
25	280	180	50	140	160

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12	No.	3/12	6/12
02	220	170	26	45	10
03	140	190	28	40	75
04	225	220	29	10	75
07	190	180	31	520	240
09	140	130	35	240	210
11	150	120	36	130	70
13	180	180	39	120	180
17	120	120	41	195	210
18	100	120	42	220	150
20	120	110	43	240	150
23	200	220	44	70	120
24	280	240	47	120	140

(b) PLACEBO TREATED GROUP

Table 10.14

Serum LH (IU/1) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12	No.	3/12	6/12
01	4.3	1.8	27	1.4	4.5
05	4.6	12.0	30	1.7	3.4
06	5.1	7.4	32	7.4	10.3
08	1.1	3.2	33	4.1	7.8
10	5.3	4.3	34	2.7	4.3
12	1.7	3.2	37	1.3	4.0
14	2.0	4.5	38	4.4	8.6
15	1.7	3.5	40	2.1	7.3
16	1.1	4.2	45	1.6	4.6
19	3.2	6.9	46	0.9	4.4
21	5.6	4.1	48	1.4	2.4
22	2.5	5.1	49	11.8	7.7
25	5.8	6.1	50	2.8	6.0

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12	No.	3/12	6/12
02	3.6	4.2	26	2.4	4.0
03	0.8	3.9	28	1.6	3.6
04	1.1	3.2	29	1.6	1.3
07	1.9	4.7	31	1.7	2.0
09	4.5	4.6	35	2.2	4.4
11	2.4	6.7	36	2.9	5.4
13	4.6	4.3	39	2.4	4.4
17	2.3	5.1	41	5.0	8.2
18	3.7	6.3	42	1.5	4.0
20	2.2	4.4	43	2.2	3.2
23	1.3	4.0	44	1.3	4.3
24	1.1	3.2	47	3.6	3.7

(b) PLACEBO TREATED GROUP

Table 10.15

Serum FSH (IU/l) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	4.2	3.1
05	7.9	3.1
06	3.4	4.5
08	1.1	1.8
10	2.2	1.2
12	1.2	0.9
14	6.5	5.6
15	4.3	4.0
16	1.2	1.9
19	6.3	6.3
21	3.6	3.3
22	0.4	0.8
25	4.2	3.5

No.	3/12	6/12
27	0.3	4.6
30	2.2	2.4
32	12.2	11.8
33	3.5	4.8
34	2.2	1.4
37	0.3	0.7
38	3.1	4.1
40	2.0	4.3
45	0.7	1.5
46	0.4	0.7
48	0.4	0.7
49	4.9	6.3
50	4.1	5.5

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	6.3	6.8
03	1.5	1.6
04	0.9	0.8
07	0.4	0.6
09	3.2	2.6
11	4.4	5.9
13	3.8	3.5
17	5.5	5.6
18	4.0	4.6
20	4.1	3.8
23	0.4	0.7
24	0.4	0.6

No.	3/12	6/12
26	6.1	6.1
28	0.9	1.3
29	3.3	0.7
31	0.4	0.6
35	1.8	2.9
36	4.5	5.2
39	4.7	4.9
41	4.4	4.8
42	3.2	4.5
43	0.9	1.0
44	2.0	2.2
47	4.2	4.1

(b) PLACEBO TREATED GROUP

Table 10.16

Serum DHT (nmol/1) in 50 Infertile Patients treated with Bromocriptine or Placebo for Six Months.

No.	3/12	6/12
01	3.8	2.3
05	3.7	3.7
06	4.7	6.7
08	2.9	2.3
10	4.9	3.1
12	5.4	6.7
14	4.5	6.1
15	5.0	3.2
16	2.8	1.6
19	3.6	3.9
21	4.3	3.2
22	3.5	7.8
25	3.4	3.5

No.	3/12	6/12
27	3.6	5.5
30	3.0	4.2
32	3.4	2.2
33	3.6	3.2
34	2.8	3.2
37	3.4	3.9
38	2.5	2.9
40	4.0	2.9
45	2.4	2.7
46	4.0	5.9
48	3.3	4.3
49	4.2	6.6
50	3.9	6.3

(a) BROMOCRIPTINE TREATED GROUP

No.	3/12	6/12
02	3.3	5.7
03	3.0	3.5
04	5.8	4.1
07	4.9	5.5
09	4.1	4.7
11	2.9	4.6
13	3.1	3.5
17	2.1	2.0
18	3.2	2.9
20	3.4	3.2
23	3.7	3.2
24	3.5	2.0

No.	3/12	6/12
26	2.5	2.7
28	3.0	4.7
29	3.4	2.5
31	4.4	3.2
35	3.8	8.9
36	3.3	8.9
39	2.5	1.4
41	3.0	2.9
42	3.0	1.4
43	7.0	9.3
44	4.0	5.9
47	4.1	4.0

(b) PLACEBO TREATED GROUP

Table 10.17

REFERENCES

REFERENCES Abbreviations of Titles based on: World List of Scientific Periodicals 1900 - 1960. Eds. Brown, P. and Stratton, G.B.
4th Edition, Butterworth, London, 1963.

and British Union - Catalogue of Periodicals, New Periodical Titles 1960 - 1968. Eds. Porter, K.I. and Koster, C.J., Butterworth, London, 1970.

BAKER, F., CRAGLE, R.G., SALISBURY, G.W. and VANDEMARK, N.L. (1957)
Spermatozoa velocities in vitro: a simple method of measurement.
Fertil. Steril. 8: 149.

BARTAK, V. (1971)
Sperm velocity test in clinical practice.
Int. J. Fertil. 16: 107.

BARTKE, A., GOLDMAN, B.D., SEX, B., DALTERIOS, S. (1977)
Effect of prolactin on pituitary and testicular function in mice with hereditary PRL deficiency.
Endocrinology. 101: 1760.

BOHNET, H.G., DALÉN, H.G., WUTTKE, W. and SCHNEIDER, H.P.G. (1975)
Hyperprolactinemic anovulatory syndrome.
J. Clin. Endocrinology. 42: 132.

BOTELLA, L. (1956)
Measurement of linear progression of the human spermatozoa as an index of male fertility.
Int. J. Fertil. 1: 113.

BRAMBLE, F.J., HOUGHTON, A.L., and JACOBS, H.S. (1975)
Proceedings: Serum follicle-stimulating hormone estimation in investigation of azoospermia.
Br. J. Surg. 62 (2): 159.

BRYANT, G.D., SILER, T.M., GREENWOOD, F.C., PASTEELS, J.L., ROBYN, C., and HUBINOT, P.O. (1971)
Radioimmunoassay of human pituitary prolactin in plasma.
Hormones 2: 139.

BOYNS, A.R. (1973) Prolactin and mammary carcinogenesis (discussion). In Human Prolactin edited by J.J. Pasteels, C. Robyn & F.J.G. Ebling, Amsterdam. Excerpta Medica, p. 263.

CARTER, J., TYSON, J., TOLIS, G., VAN VLIET, S., FAIMAN, C., and FRIESEN, H.G. (1978) Prolactin-secreting tumours and hypogonadism in 22 men. N. Engl. J. Med. 16: 857.

COCKETT, A.T., NETTO, I.C.V., DOUGHERTY, K.A. and URRY, R.L. (1975) Seminal Analysis: A review of samples from 225 men seen at an infertility clinic. J. Urol. 114: 561.

DAMASSA, D.A., KOBASHIGAWA, D., SMITH, E.R. and DAVIDSON, J.M. (1976) Negative feedback control of LH by testosterone: a quantative study in male rats. Endocrinology. 99: 736.

DAVIES, A.J. (1976) Gonadotrophine-induced changes in the Sertoli cells of the immature mouse testis. J. Reprod. Fertil. 47: 83.

DE KRETSER, D.M. (1974) The management of the infertile male. Clinics in Obstetrics and Gynaecology. 1 (2): 409.

DE KRETSER, D.M., BURGER, H.G., FORTUNE, D., HUDSON, B., LONG, A., PAULSEN, C.A. and TAFT, H.P. (1972) Hormonal, histological and genetic studies on male infertility. J. Clinical Endocrinology and Metabolism. 35: 392.

DE KRETSER, D.M., BURGER, H.G., and HUDSON, B. (1974). Aspects of male infertility: In: proceedings of Serono Symposium on Male Reproduction. Ed. R.E. Mancini and L. Martini. N.Y. Academic Press.

DOTT, H. and WALTON, A. (1953) Apparatus for studying semen metabolism and sperm motility under constant fluid perfusion and known gaseous partial pressures. J. Physiol. 122: 1, P.

ELIASSON, R. (1971a)
Standards for investigation of human semen.
Andrologie. 3: 49.

ELIASSON, R. (1971b)
Inter-laboratory co-ordination and control in andrology.
Andrologie. 3: 113.

FARRIS, E.J. (1949)
The number of motile spermatozoa as an index of fertility in man: a study of 406 semen specimens.
J. Urol. 61: 1099.

FOSSATI, P., ASFOUR, M., BLACKER, C., BOUTEMY, J.J. and HERMAND, E. (1979)
Serum and seminal gonadotropins in normal and infertile men: correlations with sperm count, prolactinemia and seminal prolactin.
Archives of Andrology. 2: 247.

FRANCHIMONT, P., MILLET, D., VENDRELY, E., LETAWE, J., LEGROS, J. and NETTER, A. (1972)
Relationship between spermatogenesis and serum gonadotrophin levels in azoospermia and oligospermia.
J. Clin. Endocrinology & Metab. 34: 1003.

FRANKS, S., JACOBS, H.S., MARTIN, N. and NABARRO, J.D.N. (1978)
Hyperprolactinemia and impotence.
Clin. Endocrinol. 8: 277.

FRANTZ, W.L. (1973)
Pharmacological factors affecting prolactin secretion (discussion).
In: Human Prolactin.
Ed. J.J. Pasteels, C. Robyn, F.J.G. Ebling, Amsterdam.
Excerpta Medica. p. 305.

FREDRICSSON, B. (1979)
Morphologic evaluation of spermatozoa in different laboratories.
Andrologia. 11 (1): 57.

FREUND, M. (1962)
Interrelationship among the characteristics of human semen and factors affecting semen specimen quality.
J. Reprod. Fertil. 4: 143.

FREUND, M. and WIEDERMAN, J. (1966)
Factors affecting the dilution, freezing and storage of human semen.
J. Reprod. Fertil. 11: 1.

FRIESEN, H., TOLIS, G., SCHUUER, R. and HAWANG, P. (1973)
Studies of human prolactin: Chemistry Radio receptorassay and clinical significance.
In: Human Prolactin.
Ed. J.J. Pasteels, C. Robyn, F.J.G. Ebling, Amsterdam.
Excerpta Medica p.11.

FRIESEN, H.G., FORNIER, P. and DESJARDIN, P. (1973)
Pituitary prolactin in pregnancy and normal and abnormal lactation.
Clin. Obstet. Gynecol. 16: 25.

GASSNER, F.X., GOLDZOEHIER, J.W., MASKEN, J.F. and HOPWOOD, M.L. (1959)
The objective measurement of sperm motility.
Fertil. Steril. 10: 488.

GEMZELL, C., KJESSLER, B. (1964)
Treatment of infertility after partial hypophysectomy with human pituitary gonadotropins.
Lancet 1: 644.

GORDON, I.M. and DANEKAR, R.V. (1977)
Fine structural localization of phosphatase activity on the plasma membrane of the rabbit sperm head.
J. Reprod. Fertil. 49: 155.

GRAY, J. (1955)
The movement of sea-urchin spermatozoa.
J. Exp. Biol. 32: 775.

HARVEY, C. (1953)
A fertility index derived from semen analysis.
J. Clin. Pathol. 6: 232.

HARVEY, C. (1960)
The speed of human spermatozoa and the effect of various diluents, with some preliminary observations on clinical material.
J. Reprod. Fertil. 1: 84.

HARVEY, C. and JOHNSON, M.H. (1945)
Assessment of male fertility by semen analysis.
Lancet, ii, 99: 134.

HEATH, H. (1963)
Plasma gonadotrophins in germinal cell aplasia.
J. Urol. 109: 847.

HOPKINSON, C.R.N., MAUSS, J., SCHENCK, B., FRITZE, E. and HIRSCHLAUSER, C. (1977) Some interrelationships between plasma levels of LH, FSH, Oestradiol 17B androgens and semen analysis data in male infertility patients. Andrologia 9: 216.

HOVATTA, O., KOSKIMIES, A.I., RANTA, T., STENMAN, U.H. and SEPPALA, M. (1979) Bromocriptine treatment of oligospermia: a double-blind study. Clin. Endocrinol. 11: 377.

HUNTER, W.M., EDMOND, P., WATSON, G.S. and McLEAN, N. (1974) J. Clin. Endocrinol. & Metab. 39: 740.

JACKAMAN, R., CHANADIAN, R., ANSELL, I.D., McLOUGHLIN, P.V.A. and CHISHOLM, G.D. (1977) Relationships between spermatogenesis and serum hormone levels in subfertile men. Br. J. Obstet. & Gynaecol. 84: 692.

JANICK, J. and MACLEOD, J. (1970) Paper: The measurement of human spermatozoan motility. Fertil. Steril. 21 (2): 140.

JOHNSEN, S.G. (1966) A study of human testicular function by the use of human menopausal gonadotrophin and of human chorionic gonadotrophin in male hypogonadotrophic eunuchoidism and infantilism. Acta Endocrinol. 53: 315.

KATZ, D.F. and DOTT, H.M. (1975) Methods of measuring swimming speed of spermatozoa. J. Reprod. Fertil. 45: 263.

KJESSLER, B. and WILD, L. (1973) Follicular-stimulating hormone (FSH) and interstitial cell-stimulating hormone (ICSH) in relation to gametic output in 643 males. Acta Endocrinol. 72: 243.

KOSKIMIES, A.I., HOVATTA, O., RANTA, T. and SEPPALA, M. (1978) Serum and seminal plasma prolactin levels in oligospermia. Int. J. Fertil. 23: 76.

KRAUSE, W. (1977) Therapeutische Beeinflussbarkeit von spermatogenese - störungen bei hyperprolaktinämie ohne hypophysenadenom. Fortschr. Med. 95: 1984.

KRUEGER, P.M., HODGEN, G.D. and SHERINS, R.J. (1974)
New evidence for the role of the Sertoli cell and spermatogonia in feedback control of FSH secretion in male rats.
Endocrinol. 94 (4): 955.

LEONARD, J.M., LEACH, R.B., COUTURE, M. and PAULSEN, C.A. (1972)
Plasma and urinary follicular-stimulating hormone levels in oligospermia.
J. Clin. Endocrinol & Metab. 34: 209.

LEWIN, L.M., MELMED, S., PASSWELL, J.H., YANIVAS, Y., BRISH, M., ORDA, S., BDICHIS, H. and BANK, H. (1978)
Myoinositol in human neonates: serum concentrations and normal handling.
Pediatr. Res. 12 (1): 3.

LOPATA, A., PATULLO, M.J., CHANG, A. and JAMES, B. (1976)
A method for collecting motile spermatozoa from human semen.
Fertil. Steril. 27: 677.

LUNENFELD, V., INSLER, A., ESHKOL, A. and BIRENBOIM, N. (1974).
Pituitary responsiveness to gonadotrophine-releasing hormone.
Hormones & Metabolic Research. Suppl. 5, 184.

MACLEOD, J. (1951)
Semen quality in one thousand men of known fertility and in eight hundred cases of infertile marriage.
Fertil. Steril. 2: 115.

MACLEOD, J. (1969)
Further observation on the role of varicocele in human male infertility.
Fertil. Steril. 20: 545.

MACLEOD, J. and GOLD, R.Z. (1951)
The male factor in fertility and infertility. II.
Spermatozoan counts in 1000 men of known fertility and 1000 cases of infertile marriage.
J. Urol. 66: 3: 436.

MACLEOD, J. and GOLD, R.Z. (1953)
The male factor in fertility and infertility. VI.
Semen quality and certain other factors in relation to ease of conception.
Fertil. Steril. 4: 10.

MACLEOD, J. and GOLD, R.Z. (1956)
The male factor in fertility and infertility, VIII. A study of variations in semen quality.
Fertil. Steril. 7: 387.

MACLEOD, J., PAZIANOS, A., RAY, B. (1966)
The restoration of human spermatogenesis of the reproductive tract with urinary gonadotrophins following hypophysectomy.
Fertil. Steril. 17: 7.

MAGRINI, G., EBINER, J.R., BURKHARDT, P. and FELBER, J.P. (1976)
Study on the relationship between plasma prolactin and androgen metabolism in man.
J. Clin. Endocrinol. Metab. 43: 944.

MAKLER, A., ZAIDISE, I., PALDI, E. and BRANDES, J.M. (1979a)
Factors affecting sperm motility. I. In vitro change in motility with time after ejaculation.
Fertil. Steril. 31 (2): 147.

MAKLER, A., BLUMENFELD, Z., BRANDES, J.M. and PALDI, E. (1979b)
Factors affecting sperm motility. II. Human sperm velocity with percentage of motility as influenced by semen dilution.
Fertil. Steril. 32 (4): 443.

MAKLER, A., ITSKOVITZ, J., BRANDES, J.M. and PALDI, E. (1979c)
Sperm velocity and percentage of motility in 100 normospermic specimens analysed by the multiple-exposure photography (MEP) method.
Fertil. Steril. 31 (2): 155.

MERINO, G., CANALES, E.S., VADILLO, M.L., FORSBACH, G., SOLIS, J. and ZARATE, A. (1980)
Abnormal prolactin levels in serum and seminal plasma in infertile men.
Archives of Andrology. 4: 353.

MILLETT, D., FRANCHIMONT, P., NETTER, J.P. (1972)
Les Stérilités masculines secrétoires d'origine testicularie.
Corrélations histohormonales FSH, LH, stéroides.
In Thibault, c(ed).
Fécondité et Stérilité du Mâle.
Pain. Masson. 119.

MITCHELL, J.A., NELSON, L. and HAFEZ, E.S.E. (1976)
Motility of spermatozoa.
In: Human Semen and Fertility Regulation in Men.
Ed. E.S.E. Hafez, C. Mosby Co., St. Louis.
p. 83.

NEGRO-VILAR, A., KRULICH, L., McCANN, S.M. (1973)
Changes in serum prolactin and gonadotrophins during the sexual development of the male rat.
Endocrinol. 93: 660.

NICHOLL, C.S. and BURN, H.A. (1972)
On the actions of prolactin among the vertebrates: is there a common denomination.
In: Lactogenic Hormones.
Foundation Symposium.
ed. G.E.W. Wolstenholme, J. Knight, Lond. Churchill Livingstone p. 299.

OJAKIAN, G.K. and KATZ, D.F. (1973).
A simple technique for the measurement of swimming speed of chlamydomonas.
Exp. Cell Res. 81: 487.

PAGE, E.W. and HOULDING, F. (1951)
The clinical interpretation of a thousand semen analyses among applicants for sterility studies.
Fertil. Steril. 2: 140.

PAULSEN, C.A. (1965)
The effect of human menopausal gonadotrophin on spermatogenesis in hypogonadotrophic hypogonadism.
In: Proceedings Pan-American Congress of Endocrinology, International Congress Series. 112. Amsterdam.
Excerpta Medica 398.

PIERREPOINT, C.G., JOHN, B.M., GROOM, G.V., WILSON, D.W. and GOW, J.C. (1978)
Prolactin and testosterone levels in the plasma of fertile and infertile men.
J. Endocrinol. 76: 171.

NAGHMA-E-REHAN, AQUILES, J., SOBRERO, J. AND FERTIG, J.W. (1974)
The semen of fertile men: statistical analysis of 1300 men.
Fertil. Steril. 26 (6): 492.

RIKMENSPOEL, R. and VAN HERPEN, G.A. (1956)
A microscopic film of the movements of bull sperm cells in dark-field illumination.
Experimentia 12: 160.

RIKMENSPOEL, R. (1957)
Photo-electric and cinematographic measurements of the "motility" of bull sperm cells.
Research Institute for Animal Husbandry. TNO No. 105. Utrecht. The Netherlands, 94.

ROSEN, S.W. and WEINTRAUB, B.D. (1971)
Monotropic increase of serum FSH correlated with low sperm count in young men with idiopathic oligospermia and azoospermia.
J. Clin. Endocrinol. & Metab. 32: 410.

ROTHSCHILD, X.X. (1949)
Measurement of sperm activity before artificial insemination.
Nature (London). 163: 358.

ROTHSCHILD, Lord (1953)
A new method of measuring sperm speeds.
Nature (London). 171: 512.

ROTHSCHILD, Lord (1953)
A new method of measuring sperm speeds.
J. Exp. Biol. 30: 312.

RUBIN, R.T., POLAND, R.E. and TOWER, B.B. (1976)
Prolactin-related testosterone secretion in normal adult men.
J. Clin. Endocrinol. & Metab. 43: 112.

SAIDI, K., WENN, R.V. and SHARIF, F. (1977)
Bromocriptine for male infertility.
Lancet. 99: 250.

SANTEN, R.J., LEONARD, J.M., SHERINS, R.J., GANDY, H.M. and PAULSEN, C.A. (1971)
Short and long-term effects of clomiphene citrate on the pituitary testicular axis.
J. Clin. Endocrinol. & Metab. 33: 970.

SANTOMAURO, A.G., SCIARRA, J.J. and VARMA, A.O. (1972)
A clinical investigation of the role of semen analysis and post-coital tests in the evaluation of male infertility.
Fertil. & Steril. 23: 245.

SCHALLY, A.V., KASTIN, A.J. and ARIMURA, A. (1972)
Am. J. Obstet. Gynec. 114: 423.

SCHOENFELD, C.Y., AMELAR, R.D., DUBIN, L. and NUMEROFF, M. (1979)
Prolactin, fructose and zinc levels found in human seminal plasma.
Fertil. Steril. 32: 2: 206.

SEGAL, S., POLISHUK, W.Z. and BEN-DAVID, M. (1976)
Hypoprolactinaemic male infertility.
Fertil. Steril. 27: 1425.

SEPPALA, M., HIRVONEN, E., RANTA, T., VIRKUNEN, P. and LEPPALUOTO, J. (1975)
Raised serum prolactin levels in amenorrhoea.
B.M.J. 2: 305.

SEPPALA, M., HIRVONEN, E., RANTA, T. and VIRKUNEN, P. (1976 a)
Bromocriptine treatment of secondary amenorrhoea.
Lancet 1: 1154.

SEPPALA, M., HIRVONEN, E., RANTA, T. and VIRKUNEN, P. (1976 b)
Hyperprolactinaemia and luteal insufficiency.
Lancet 1: 229.

SHAH, G.V., DESAI, R.B., and SHETH, A.R. (1976)
Effect of prolactin on metabolism of human spermatozoa.
Fertil. Steril. 27: 1292.

SHETH, A.R., MUGATWALA, P.P., SHAH, G.V. and RAO, S.S. (1975)
Occurrence of prolactin in human semen.
Fertil. Steril. 26: 905.

SHETH, A.R., GUNJIKAR, A.N. and SHAH, G.V. (1979)
Effect of LH, prolactin and spermine on ATPase activity of human spermatozoa.
Andrologia 11 (1): 11.

SOKOLOSKI, J.E., BASCO, L., STOREY, B.T., WOLF, D.P. (1977)
Turbidimetric analysis of human sperm motility.
Fertil. Steril. 28 (12): 1337.

SPERA, G., RE, M., WEITZ, R., MONTAGUE, G., IANNITELLI, M. and FRAIOLI, F. (1978)
Relationship between plasma levels of FSH and impairment of spermatogenesis in male infertility.
In: Recent Progress in Andrology. Ed. A. Fabbrini & E. Steinberger. Academic Press. p. 377.

SUOMINEN, J.J.O., NIKKANEN, V., MULTAMAKI, S. and HYYPPA, M.(1979)
Prolactin in azoospermic men and its relationship to testicular morphology, serum testosterone and gonadotrophin levels.
Andrologia 11 (1): 15.

THORNER, M.O., McNEILLY, A.S., HAGAN, C. and BESSER, G.M. (1974)
Long-term treatment of galactorrhoea and hypogonadism with bromocriptine.
B.M.J. 419.

TOLIS, G., WILSON, G., KLEISSL, P., NAFTOLIN, F. and POSNER, B. (1979)
Seminal fluid prolactin: studies in normal subjects and hypergonadotropic oligospermic.
Fertil. Steril. 32: 96.

TREVELYAN, W.E., PROCTOR, D.P. and HARRISON, G.S. (1950)
Detection of sugars on paper chromatography.
Nature 166: 444.

VAN ZYL, J.A. (1972)
Oligozoospermia: a seven-year survey of the incidence, chromosomal aberrations, treatment and pregnancy rate.
Int. J. Fertil. 20: 129.

REFERENCES Abbreviations of Titles based on: World List of Scientific Periodicals 1900 - 1960. Eds. Brown, P. and Stratton, G.B. 4th Edition, Butterworth, London, 1963.

and British Union - Catalogue of Periodicals, New Periodical Titles 1960 - 1968. Eds. Porter, K.I. and Koster, C.J., Butterworth, London, 1970.

ARAGONA, C., BOHNET, H.G., FRIESEN, H.G. (1977)
Localization of prolactin in prostate and testis: the role of serum prolactin concentration on the testicular LH receptor.
Acta Endocrinol. (kbh). 84: 402.

BISWAS, S., FERGUSON, K.M., STEDRONSKA, J., BAFFOE, G., MANSFIELD, M.D., and KOSBAB, M.H. (1978)
Fructose and hormone levels in semen: their correlations with sperm counts and motility.
Fertil. Steril. 30: 200.

CARTER, J.N., TYSON, J.E., TOLIS, G., VAN CLICT, C., FAIMAN and FRIESEN, H.G. (1978)
Prolactin secreting tumors and hypgonadism in 22 men.
N. Engl. J. Med. 299: 847.

CHARREAU, E.H., ATTRAMADAL, A., TORJESEN, P.A., PURVIS, K., CALANDRA, R. and HANSSON, V (1977)
Prolactin-binding in rat testis: specific receptors in interstitial cells.
Molec. Cell. Endocrinol. 6: 303.

CHEMES, H.E., PODESTA, E. and RIVAROLA, M.A. (1976)
Action of testosterone, dihydrotestosterone and 5 alpha-androstane-3 alpha, 17β-diol on the spermatogenesis of immature rats.
Biol. Reprod. 14: 332.

DE ALOYSIO, D., D'URSO, N., GARDINI, G., NICOLETTI, G. and GORI, G. (1978)
Plasma and Semen Androgens in Normospermic and Dysspermic Subjects.
Acta Europaea Fertilitatis. 9: (3) 139.

DE ALOYSIO, D., BUSACCHI, P. and BOLELLI, G.F (1974)
Radioimmunological assay of testosterone and gonadotropins in plasma and seminal fluid of healthy subjects and patients with dysspermia.
Acta Europaea Fertilitatis. Vol. 5 n. 4.

DE JONG, F.H., UILEMBROOK, J.T.J. and van der MOLEN, H.J (1975)
Oestradiol-17β, testosterone and gonadotrophins in oestradiol-17β-treated intact adult male rats.
J. Endocrinol. 65: 281.

DORRINGTON, J.H., FRITZ, I.B. and ARMSTRONG, D.T (1978)
Int. J. Androl. suppl. no. 2 pp 53-65.

FIORELLI, G., BONELLI, D., FORTI, G., GONNELI, P., PAZZAGLI, M. and SERIO, M (1976)
Simultaneous determination of androstenedione, testosterone and 5 alpha-dihydrotestosterone in human spermatic and peripheral venous plasma.
J. Ster. Biochem. 7: 113.

FRAIOLI, F., PAOLUCCI, F., DONDERO, G., SPERA, G. and ISIDORI, A (1980)
Prolactin secreting adenoma in man and the role of prolactin in spermatogenesis.
J. Endocrinol. Invest. 3: 155.

FRANCHIMONT, P., CHARI, S. and DEMOULIN, A (1975)
J. Reprod. Fertil. 44: 335.

GAY, V.L (1976)
Species variation in the metabolism of dihydrotestosterone: correlation with reported variations in behavioral response (abstra. 23).
In: Program of the Ninth Meeting of the Society for the Study of Reproduction. Philadelphia, Aug. 10-13, p. 27.

HARRIS, M.E., BARTKE, A., WEISZ, J. and WATSON, D (1977)
Effects of testosterone and dihydrotestosterone on spermatogenesis, rat testis fluid, and peripheral androgen levels in hypophysectomized rats.
Fertil. Steril. Vol. 28 No. 10.

JEQUIER, A.M., CRICH, J.C. and ANSELL, I.D. (1979)
Clinical findings and testicular histology in three hyperprolactinemic infertil e men.
Fertil. Steril. 31: 525.

LABRIE, F., LAGACÉ, L., FERLAND, L., KELLY, P.A., DROUIN, J., MASSICOTTE, J., BONNE, C., RAYNAUD, J.P. and DORRINGTON, J.H (1978)
Int. J. Androl. suppl. no. 2. pp 81-101.

LEE, D.K.H., BIRD, C.E., CLARK, A.F (1975)
In vivo metabolism of ^{3}H-testosterone in adult male rats: effects of estrogen administration.
Steroids 26: 137.

LE LANNOUR, D., MASSART, C., CHAMBOU, Y., NICOL, M. and ALLANNIC, H (1980) Testosterone and 5 alpha-dihydrotestosterone concentrations in human seminal plasma. Int. J. Andrology. 3: 502.

L'HERMITE, M.H., WAGNER, K., BOCKEL, W., WIEGELMANN, C., HIRSCHHAUSER and NIESCHLAG, E (1976) Human prolactin in male infertility. In: Sperm Action Progr. Reprod. Biol. Vol. 1. Basel: Karger, pp 157-161.

MARYNICK, S.P., HAVENS, W.W. II, EBERY, M.H., LORIAUX, D.L. and ROBERTSON, S (1976) Studies on the transfer of steroid hormones across the blood-cerebrospinal fluid barrier in the rhesus monkey. Endocrinology 99: 400.

MULDER, E., PETERS, M.J., van BEURDEN, W.M.O., GALDIERI, M., ROMMERTS, F.F.G., JANSZEN, F.H.A. and van der MOLEN, H.J (1976) Androgen receptors in isolated cell preparations obtained from rat testicular tissue. J. Endocrinol. 70: 331.

NIERMANN, H., LENAU, H., KOBERG, A. and KOBERG, B (1977) Serum-Prolaktinspiegel bei verschiedenen Krankheitsbildern in der Andrologie. In: Fortschritte der Fertilitatsforschung, V.K., Semm, H. Rausher, C. Schirren (Hrsg) Berlin, Grosse, pp 143-146.

PEDRON, N., GINER, J., HICKS, J.J., ROSADO, A (1975) Comparitive glycolytic metabolism of sperm from normal, asthenospermic and oligoasthenospermic men. Fertil. Steril. 26: 309.

PEDRON, N., GINER, J. (1978) Effect of prolactin on the glycolytic metabolism of spermatozoa from infertile subjects. Fertil. Steril. Vol. 29 No. 4 pp 428-430.

PURVIS, K., BRENNER, P.F., LANDGREN, B.M., CEKAN, Z. and DICZFALUSY, E (1975 Indices of gonadal function in the human male. I - Plasma levels of inconjugated steroids and gonadotrophins under normal and pathological conditions. Clin. Endocrinol. 4: 237.

PURVIS, K., LANDGREN, B.M., CEKAN, Z. and DICZFALUSY, E (1975) Indices of gonadal function in the human male. II - Plasma levels of steroids in normal and pathological conditions. Clin. Endocrinol. 4: 247.

PURVIS, K., CALANDRA, R. and HANSSON, V (1977)
In: Martini, L. & Motta, M. ed.
Androgens and antiandrogens,
pp 37.
Raven Press, N.Y.

RIVAROLA, M.A., PODESTA, E.J., CHEMES, H.E., CIGORRAGA, S (1975)
Androgen metabolism in the
seminiferous tubules.
In: Hormonal Regulation of
Spermatogenesis.
Ed. F.S. French, V. Hansson,
E.M. Ritzen, S.N. Nayfeh,
N.Y. Plenum Press. p. 25.

RJOSK, H.K. and SCHILL, W.B. (1979)
Serum prolactin in male infertility.
Andrologia 11: 297.

ROULIER, R.A., MATTEI, and FRANCHIMONT, P (1978)
Prolactin in male reproductive
functions.
In: Progress in prolactin
physiology and pathology.
C. Robyn, M. Harter (Eds).
Elsevier/n. Holland, Biomed. Press.
pp 305-316.

SETCHELL, B.P. and MAIN, S.J (1975)
The blood-testis barrier and steroids.
In: F.S. French (Ed).
Hormonal Regulation of Spermatogenesis.
p 223. Plenum Press, N.Y.

SHAH, G.V., DESAI, R.B. and SHETH, A.R (1976)
Effect of prolactin on metabolism
of human spermatozoa.
Fertil. Steril. 27: 1292.

SHOLITON, L.J., SRIVASTAVA, L., TAYLOR, B.B.(1975)
The in vitro and in vivo effects of
diethylstilbestrol on testicular
synthesis of testosterone.
Steroids 26: 797.

SMITH, M.L., WIJDAN, A., LUQMAN, J. and RAKOFF, S (1979)
Correlations between seminal
radioimmunoreactive prolactin, sperm
count and sperm motility in
prevasectomy and infertility clinic
patients.
Fertil. Steril. 32: 3.

STEINBERGER, E (1975)
Hormonal regulation of the
seminiferous tubule function.
In: Hormonal Regulation of
Spermatogenesis, Ed. F.S. French,
V. Hansson, E.M. Ritzen, S.N. Nayfeh,
N.Y. Plenum Press, p. 337.

TAMPION, D., GIBBONS, R.A (1963) Swimming rate of bull spermatozoa in various media and the effect of dilution. J. Reprod. Fertil. 5: 259.

TRESGUERRES, J.A.F., ESQUIFINO, A.I., PEREZ MENDEZ, L.F. and LOPEZ-CALDERON, A. (1980) Possible role of prolactin in the inhibitory effect of estradiol on the hypothalamic-pituitary-testicular axis in the rat. Endocrinol. Vol. 108 n. 1 p 83-87.

VAN BEURDEN, W.O., MULDER, E., DE JONG, F.H. and van der MOLEN, H.J (1977) The effects of estrogens on LH plasma levels and on T production in intact and hypophyectomized rats. Endocrinol. 101: 342.

VAN DOORN, E.J., BURNS, B., WOOD, D., BIRD, C.E. and CLARK, A.F (1975) In vivo metabolism of ^{3}H-androstanediol in adult male rats. J. Steroid Biochem. 6: 1549.