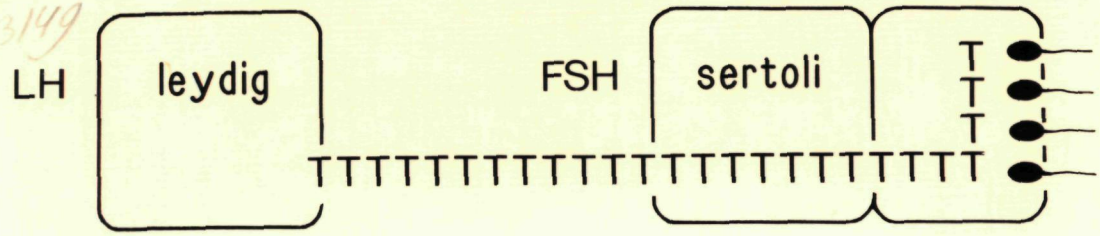
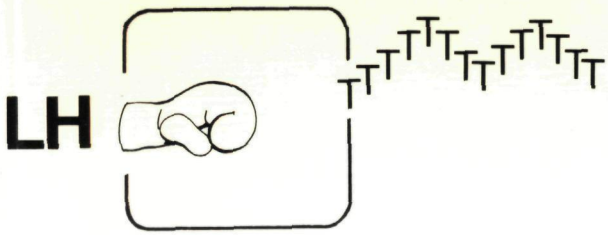
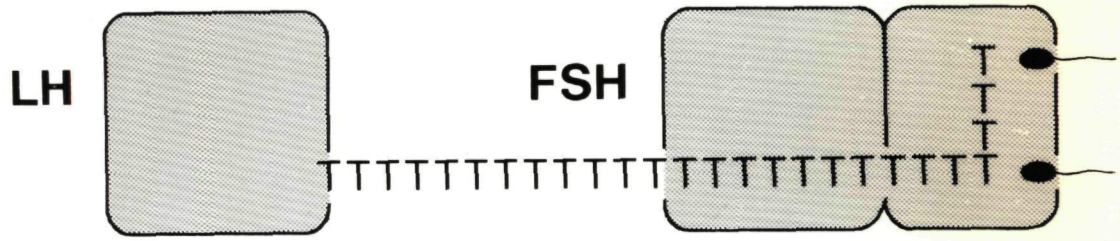


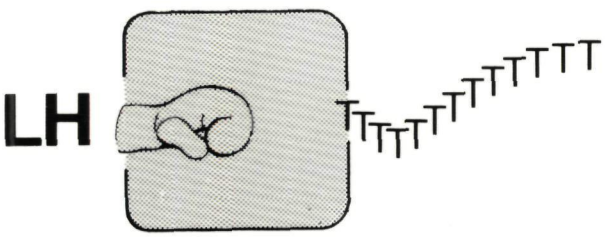


J.M.J. DONY

IDIOPATHIC OLIGOSPERMIA

endocrinological and therapeutical aspects





IDIOPATHIC OLIGOSPERMIA

Endocrinological and Therapeutical Aspects

IDIOPATHIC OLIGOSPERMIA

Endocrinological and Therapeutical Aspects

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE GENEESKUNDE AAN DE
KATHOLIEKE UNIVERSITEIT TE NIJMEGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR J.H.G.I. GIESBERS
VOLGENS BESLUIT VAN HET
COLLEGE VAN DEKANEN IN HET
OPENBAAR TE VERDEDIGEN OP
VRIJDAG 14 JUNI 1985
DES NAMIDDAGS TE 4 UUR

DOOR

JULIEN MARIE JOSEPH DONY
GEBOREN TE PRINCENHAGE-BREDA

1985

krips repro meppel

PROMOTOR : Prof. Dr R. Rolland
CO-REFERENT : Dr A.G.H. Smals

The studies presented in this thesis were performed in the Division of Endocrinology and Infertility of the Department of Gynaecology (head: Prof. dr R. Rolland) and in the Division of Endocrinology (head: Prof. dr P.W.C. Kloppenborg) of the Department of Medicine, Sint Radboud Hospital, University of Nijmegen, The Netherlands.

TOUT VIENT A POINCT
QUI PEUT ATTENDRE . . . ANNETTE!
(Rabelais, Pantagruel IV, 48)

ABBREVIATIONS

ABP	:	androgen binding protein
A.M.	:	ante meridiem (before noon)
CBG	:	cortisol binding globulin
E_2	:	estradiol
FSH	:	follicle stimulating hormone
hCG	:	*human chorionic gonadotropin*
HSD	:	hydroxysteroid dehydrogenase
LH	:	luteinizing hormone
LH-RH	:	luteinizing hormone-releasing hormone
max.	:	maximal
mg	:	milligram (10^{-3} gram)
mis	:	meiosis including substance
mIU	:	milli international units
mps	:	meiosis preventing substance
µg	:	microgram (10^{-6} gram)
N	:	number of subjects
ng	:	nanogram (10^{-9} gram)
17-OHP	:	17-hydroxyprogesterone
P	:	progesterone
pg	:	picogram (10^{-12} gram)
P.M.	:	post meridiem (after noon)
PRL	:	prolactin
RIA	:	radioimmunoassay
SD	:	standard deviation
SE	:	standard error
SEM	:	standard error of the mean
SHBG	:	sex hormone binding globulin
T	:	testosterone
t	:	time
TAM	:	tamoxifen
TL	:	Δ^1-testolactone

oligospermia : oligozoospermie

CONTENTS

CHAPTER I

INTRODUCTION

Infertility is no longer thought a woman's fault, as men alone account for approximately 30 per cent of all cases of infertility and play a contributing role in another 20 per cent (1,2). Although male infertility can be caused by psychological or physical ejaculatory dysfunction, this occurs only sporadically and impairment of sperm quality is by far more the most frequent. The percentage of allegedly fertile men with sperm counts below 20 x 10^6/ml varies between 5 and 23 per cent (table I) (3-12). The fertility of the oligospermic man's wife will often compensate for his subfertility (13,14). In support of this assumption is the experience that the wife of an azoospermic man conceives much easier with donor insemination than the wife of an oligospermic man (15). Due to the frequent compensation by the partner's fertility only a part of the male population with oligospermia asks for medical evaluation of its subfertility.

In this study oligospermia is defined according to MacLeod and Gold (4). Their definition is based on single sperm counts of 1000 men whose wives had registered at an infertility clinic. Five per cent of these men had a sperm count below 20 x 10^6/ml. Arbitrarily this level was chosen to differentiate between fertile and infertile men. However, in later publications this sperm standard was challenged by several authors (5,8,9,12). Their studies were mostly based on single sperm counts of prevasectomy patients. The results of different studies are summarized in table I. The selection of the patient population was different in these studies and probably contributed to the wide range of the results. Since the proof of fertility was the strongest in the study of MacLeod and Gold (4), their conclusions seem the most valid and therefore have been accepted by most investigators.

Although we also use the criterium of MacLeod and Gold (4), the diagnosis oligospermia is only made if the sperm count is persistently lower than 20 x 10^6/ml in at least three ejaculates, each obtained after sexual abstinence for 2-5 days (16). The minimum standard of three pretreatment sperm analyses was chosen on the analogy of the studies of most investigators and also for practical reasons.

Table I: Comparison of sperm density cohorts in different series of fertile men

Authors	year of publication	number of subjects	mode of recruitment	mean volume (ml)	mean count (10^6/ml)	% of counts (10^6/ml) ≤10 (A)	10.1-20 (B)	≤20 (A + B)
Hotchkiss et al.[3]	1938	100	prenatal*	3.36	137			1
Macleod & Gold [4]	1951	1000	prenatal*	3.40	107	2.0	3.0	5
Nelson & Bunge [5]	1974	386	prevasectomy	2.83	48	4.7	15.5	20.2
Rehan et al. [6]	1975	1300	prevasectomy	3.20	79	1.9	5.1	7
Sobrero & Rehan [7]	1975	100	prenatal*	3.31	81			5
Smith & Steinberger[8]	1977	2000	prevasectomy	2.50	70	9.3	9.6	19.9
Zukerman et al.[9]	1977	4122	prevasectomy		63	11.7	11.2	22.9
David et al. [10]	1979	190	prevasectomy		98	3.2	3.7	6.9
Jouannet [11]	1981	484	prevasectomy cryopreservation donor selection	3.6	93			8
de Castro et al.[12]	1983	598	prevasectomy			7.5	14.5	22

* prenatal = husbands of wives visiting the prenatal clinic

However it has to be mentioned that in euspermic men there exists a circannual rhythm in sperm count which accounts for approximately 34% of the variance during the year (17) and may in part explain the within subject variability. The within subject coefficient of variation for sperm count is in the order of 40% and although the period of abstinence is an important factor, the greater part of the within subject variability remains unexplained (18). Therefore some authors advise a minimum of 5-6 pretreatment semen analyses if the purpose of the study is to investigate the effect of treatment (19,20).

Abnormalities in sperm density are mostly seen in combination with impairment of both sperm motility and morphology in infertile men (4,21). As sperm density is easy to determine in contrast to motility and morphology, most studies about oligospermia refer only to this parameter. However, sperm motility and morphology are considered the most crucial factors in determining the fertilizing capacity (22). Therefore, men with normal motility and morphology but with low sperm density are rarely seen at the andrologic clinic. Experience from in vitro fertilization programs shows that the incubation medium has to contain at least 0.2 million sperms/ml with good motility (23). This finding relativates at least the phrase "only one spermatozoon is necessary for conception" and supports the relevance of sperm density for fertilization. Besides during the migration of sperm through the female genital tract an impressive reduction in sperm density and selection of sperm morphology takes place. The final number of spermatozoa in the ampulla of the oviduct is estimated to be less than 200. The contrast between this number and the minimum number necessary for in vitro fertilization can be explained by the supposition that spermatozoa reaching the site of fertilization, represent a highly selective sample of extreme fertilizable gametes (24).

All men investigated in this study showed besides oligospermia also impairment of sperm motility and morphology. Since motility is the most crucial parameter for the assessment of the potential fertility of sperm, its determination needs special attention. The sperm motility determinations in this study were performed as soon as liquifaction was completed. This was generally the case within 30 minutes after sperm collection. The motility was determined mostly by the same experienced investigator. Since the vesicular secretions have an adverse effect on sperm motility (25) it is important especially in case of oligospermia, that motility determina-

tion takes place immediately after liquifaction.

Although not mentioned in this study, proof of deficient motility was also obtained by evaluation of the in vivo and in vitro ability to penetrate cervical mucus. The first was done with post coital testing and the last by means of the sperm migration test of Kremer (26). In this test the ability of sperm to migrate from a sperm pool in a glass rod filled with cervical mucus is studied. The results of both tests were poor or negative for all the patients in the pretreatment period.

Etiology of oligospermia

Oligospermia has multiple causes. The subdivision in pretesticular, testicular and post-testicular causes, mostly used in case of azoospermia is only of limited value for oligospermia. Table II summarizes the major causes of oligospermia. Depending on the severeness of the underlying disease the same cause can result in azoospermia, different degrees of oligospermia or be compatible with eupermia. In the vast majority of moderately oligospermic men - this implies sperm density in the range of 5-20 x 10^6/ml - no definite cause can be detected (27,28) and the final diagnosis is "idiopathic oligospermia". General agreement exists as to most of the criteria necessary for this diagnosis. These criteria are summarized in table III.

The necessity to perform a testicular biopsy and/or chromosomal analysis before making the diagnosis of idiopathic oligospermia is controversial. Since both diagnostic procedures with regard to the patients were omitted in this study, this requires a justification. Before the introduction of quantitative scoring techniques of testicular biopsies, the histologic descriptions correlated poorly with sperm analysis and were of very limited value. After the introduction of (semi)quantitative studies on the morphology of testicular biopsies, several authors (29-33,72) reported on the remarkable correlation between semen analysis and the histological picture. From these studies it became clear that irreversible disorders like maturation arrest and hyalinization of the tubular wall were in fact only found in patients with severe oligospermia (< 5 x 10^6/ml). However, in patients with mild oligospermia (5-20 x 10^6/ml) and FSH levels in the normal range, the most common finding, sperm maturation was decreased and particularly disorganization of the seminiferous epithelium was pronounced.

Table II: Causes of oligospermia

- Chromosomal disorders
- Hypothalamic-pituitary disorders
- Hypo- and hyperthyroidism
- Congenital adrenal hyperplasia
- Polyglandular failure
- Metabolic disorders; chronic liver disease; uremia
- Gross obesity
- Paraplegia
- Myotonic dystrophy
- Intoxications
- Drugs (alkylating agents-salazopyrine-cimetidine-antiandrogens-estrogens-spironolactone-pesticides)
- Irradiation
- Disturbed testicular descent
- Varicocele
- Post-testicular partial obstruction
- Male infertility syndrome (androgen receptor deficiency)
- Infections: orchitis, epididymitis, prostatitis
- Immunologic disorders

- **Idiopathic oligospermia**

Because of the close correlation reported by different authors using sophisticated techniques one can question whether testicular biopsy in case of moderate oligospermia has additional value. However, one serious argument is in favour of this that the oligospermia may be caused by an obstruction in the ductal system. A study in several hundred oligospermic men demonstrated such a obstruction in only 20% of the severe oligospermic cases (34). Hence, it was concluded that in a population of moderately oligospermic men with serum FSH levels in the normal range, the value of testicular biopsy is limited to justify this as a routine procedure. Moreover, testicular biopsy carries the risk of temporary damage of the seminiferous epithelium (35) and induction of sperm antibodies (36).

Table III: Criteria for the diagnosis of idiopathic oligospermia

- Persistent sperm concentration of $<20 \times 10^6$/ml or total sperm count $<40 \times 10^6$/ml in at least 3 semen analyses
- No history or evidence on physical examination of cryptorchidism, orchitis, epididymitis or prostatitis
- No systemic disease and no intoxications
- Normal libido and potency
- Testis volume ≥ 17 ml (40)
- Varicocele ruled out by clinical investigation
- Serum FSH, -LH, -testosterone and -prolactin in the range of eupermic men

* Chromosomal anomalies ruled out by chromosomal analysis
* Intratesticular gross pathology and/or post-testicular partial obstruction ruled out by testicular biopsy

* facultative criteria

Comparable arguments have favoured the rejection of chromosomal analysis in the patients investigated in this study. In several studies (37-39) it was shown that among azoospermic men 13-15% were chromosomally abnormal, the vast majority (80%) being 47,XXY chromatin positive males. However, in oligospermic men the incidence of chromosomal anomalies was not higher than 3%, half of which was caused by anomalies of sex chromosomes and the other half by anomalies of autosomes. Since anomalies of sex chromosomes are almost always seen in combination with hypogonadism, severe oligospermia and elevated serum FSH levels, and since none of the patients in this study have shown the above features, this possibility was disregarded. The chance of 1.5%, that the oligospermia could be caused by an autosomal anomaly and therefore should not be considered as idiopathic, was considered too low to justify chromosomal analysis in all patients.

High intratesticular testosterone levels are considered to be a requisite to the completion of spermatogenesis (41). For many years the current dogma has been that in patients with disrupted spermatogenesis Leydig cell function is normal (42). However, in many of the patients with severe testicular damage, such as germ cell arrest, Sertoli cell only syndrome and seminiferous tubule hyalinization, elevated LH and low testosterone levels are present (43-56).

Further evidence of abnormalities in Leydig cell function in men with seminiferous tubular damage has been adduced from the subnormal response of testosterone to stimulation with human chorion gonadotropin or LH-RH (42,44,50,57-60). In some instances LH levels are high normal or elevated despite normal levels of testosterone, which led to the postulate that a state of compensated Leydig cell failure exists in these men. Elevation of the LH response to LH-RH in men with Sertoli cell only syndrome or Klinefelter's syndrome further substantiates these abnormalities in Leydig cell function (42,44,60,61). This supports the concept of decreased feedback from Leydig cells in this disorder. In idiopathic oligospermia there is also growing evidence, that the testicular abnormality is not limited to the seminiferous tubule but involves the Leydig cell as well. Reduced plasma testosterone levels have been found in patients with low sperm counts (42,44,51,52,56,61-68) in the presence of high normal or elevated serum LH levels (51,52,56) and increased responses to LH-RH in more than 40% of these patients (15,64,65,68-71). Furthermore, reverse correlations between basal serum LH and sperm counts have been reported by some investigators (42,46,56,72).

In addition to these abnormalities in circulating LH and testosterone levels in idiopathic oligospermia, preliminary reports from in vivo and in vitro studies point to an accumulation of 17-OHP over testosterone under basal condition (54,73-76) and after HCG stimulation (57,59,73,77,78). This favours the concept of the presence of an enzymatic defect at the 17,20-lyase step. Furthermore, in patients with idiopathic oligospermia abnormal estrogen handling has been postulated in the pathogenesis of Leydig cell dysfunction. In normal men the testes secrete estrogens which probably play a role in the local control of Leydig cell function (53,79, 80). In men with secretory azoospermia and in Klinefelter's syndrome (53,

59,75,81,82) elevated serum estradiol levels have been found. In men with idiopathic oligospermia also an increase in testicular intratubular concentration and/or an inordinate sensitivity to its inhibitory effect have been postulated (75,79,81,83) providing a rationale for the treatment of these patients with antiestrogens and/or aromatase inhibitors. Apart from defects in testosterone and/or estradiol handling, abnormalities in testicular FSH receptors (84) and androgen receptors (85-86) have been reported in infertile men. Some authors have claimed indirect and direct evidence for androgen resistance in 10 to 40% of patients with azoospermia and severe oligospermia (85,86), but this finding has been challenged by others (87).

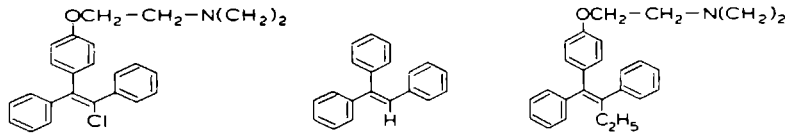
Decreased pulse frequency of LH-RH and by inference of LH very recently has been adduced as the cause of selective elevation of FSH in some patients with idiopathic oligospermia ("slow pulsing oligospermia") (88-91), localizing the cause of the spermatogenic derangement at the hypothalamic pituitary level. Increasing this pulse frequency to a normal rate by pulsatile LH-RH administration, resulted in a fall of serum FSH levels and an increase in sperm density (90).

From all these data it is evident that idiopathic oligospermia is not a distinct nosological entity but rather a mixture of hypothalamic, pituitary and gonadal abnormalities leading to spermatogenic damage, decreased sperm density and impairment in both the morphology and motility.

Therapy in idiopathic oligospermia

Ample therapeutic modalities have been used in idiopathic oligospermia, most of them based on wrong assumptions or empiricism. Examples of such therapeutic regimens are vitamine E (92,93), corticosteroids (94-96), thyroid hormone therapy (97), arginine (98-100), kallikrein (101), bromocriptine (102-104), gonadotropins (105-108), mesterolon (109,110), testosterone rebound therapy (111-113) and LH-RH (114-116). For review see Schill and Michalopoulos (117). In most of these older studies conception rates and sperm analyses were the only mentioned parameters and no hormonal data were given. This changed with the introduction of clomiphene citrate as a mode of treatment. This triphenylethylene derivate (fig. 1), a racemic mixture of two components with both estrogenic and antiestrogenic properties stimulates the secretion of pituitary gonadotropins and

thereby gonadal steroid secretion (118-124).



Clomiphene Triphenylethylene Tamoxifen

Figure 1

Although the mode of action at the hypothalamic-pituitary level is not completely understood, most of the data available suggest an antiestrogenic action at the level of the hypothalamus and an estrogenic action at the pituitary level (125).

Jungck et al. (126) was the first to report on the effect of clomiphene citrate on the spermatogenesis in normal volunteers as well as in oligospermic men. This paper was followed by numerous reports of many authors using different doses of clomiphene and regimens. After the report of Heller et al. (127) in 1969, demonstrating the toxic effect of high doses (400 mg daily) on spermatogenesis of clomiphene citrate, the impetus as to the use of clomiphene in men was accelerated by the publications of Paulson et al. (128-131) which stressed the importance of the use of low dosage and reliance on FSH determinations. Table IV summarizes the results of the most important studies. Interpretation of the different results is hampered by lack of universal agreement as to what constitutes a positive response to therapy. For example no clear-cut standard has been developed for what means a 'significant' improvement in semen analysis. Furthermore, the selection criteria of patients were inconsistent. One of the scarce double blind cross-over studies with clomiphene citrate showed a significant increase in sperm density during the treatment period whereas there was no change during the placebo period (139). The erratic results of the treatment of oligospermic men with clomiphene citrate and the assumption of a direct negative effect of the estrogenic component of clomiphene citrate on spermatogenesis led to treatment with cis-clomiphene citrate, which is devoid of estrogen action. However, even with cis-clomiphene results were erratic and unpredictable (142-144).

Table IV: Literature data on the effect of clomiphene citrate treatment in oligospermia

authors	year of publication	clomiphene citrate dose (mg/daily)	duration of treatment (months)	number of patients	number of patients showing improvement in sperm density	number of pregnancies	remarks*
Jungck et al.[126]	1964	50 25	2	20 9	13 4	not reported	
Mellinger, Thompson[132]	1966	50	20	13	10	not reported	
Mroueh et al.[133]	1967	50	1.5	15	0	0	
Palti[134]	1970	100 50 25 12.5	1 - 2	16 40 4 9	4) 15) 0) 4)	5	
Foss et al.[135]	1973	100*	0.5	114	not reported	19	controlled trial 100 mg/day for 10 days periods every months during 3 months
Schellen & Beek[136]	1974	50	1.5 - 4	101	61	19	
Paulson & Wacksman[129]	1976	25*	9 - 12	35	31	8	25 mg/day in 25 days cycles with 5 day rest period
Epstein[137]	1977	100*	4	16	10	not reported	100 mg 3 times a week
Charny[138]	1979	50 25	3 - 9	44) 12)	11	) 5)	
Rönnberg[139]	1980	50	3	30	sign.improv.	3	controlled trial
Ross et al.[140]	1980	100*	6 - 15	53	35	14	100 mg 3 times a week
Wang et al.[141]	1983	25 50	6 6	11 18	6 6	4 4	controlled trial

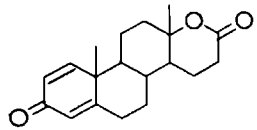
In 1976 Comhaire (145) was the first to report on the favourable results of another triphenylethylene derivative, tamoxifen, in the treatment of idiopathic oligospermia (see fig. 1). Administration of this competitive estrogen antagonist to oligospermic men resulted in a moderate increase of serum LH and FSH levels and doubling of serum testosterone levels. Sperm density doubled in his group of patients with moderate oligospermia. Interestingly this drug had already been tested extensively in the palliative treatment of breast cancer (146). Whereas in the human tamoxifen has nearly pure antiestrogenic properties (147), the reverse holds true for rodents. In rats tamoxifen causes a severe regression of both the seminal vesicles and prostate and also testicular atrophy (148). This observation may explain the long delay between registration of tamoxifen for palliative treatment in breast cancer patients and further research to test its usefulness as a potential therapeutical agent in the treatment of idiopathic oligospermia. The argument to consider tamoxifen more appropriate for the treatment of idiopathic oligospermia, came from the hypothesis that clomiphene by means of its estrogenic action could exert a direct negative effect on the seminiferous epithelium counteracting the stimulating effect of FSH and testosterone. After the first report of Comhaire several papers have been published stressing the beneficial effect of tamoxifen in doses varying from 20-30 mg daily (145, 149-154). Doubling of the sperm density and a 20-70% conception rate was reported by some authors after a treatment period of 3 to 12 months. Table V summarizes the data of these studies. Willis (149) however using a much lower dose of only 10 mg could not observe any effect at all on sperm parameters despite clear-cut changes in serum gonadotropin, testosterone and estradiol levels. These data suggested that the effect of tamoxifen on spermatogenesis is dose dependent. Otherwise, the severity of the oligospermia in most of the patients in the study of Willis could also at least in part explain the lack of sperm improvement. The disadvantage of tamoxifen and also cis-clomiphene treatment in idiopathic oligospermia may be due to the fact that both agents bring about an increase in serum estradiol levels comparable or even greater than that of testosterone (145,149, 150). Therefore both agents produced either no change or even a decrease in the testosterone/estradiol ratio.

As already mentioned, estradiol probably plays a role in the regulation of spermatogenesis, first by direct action on the seminiferous tubules

Table V: Literature data on the effect of tamoxifen treatment in idiopathic oligospermia

Authors	year of publication	tamoxifen dose (mg/daily)	duration of treatment (months)	number of patients	number of patients showing improvement in sperm density	number of pregnancies
Comhaire[145]	1976	2 x 10	6 - 11	15	13	3
Willis et al.[149]	1977	1 x 10	6	9	1	1
Vermeulen & Comhaire[150]	1978	2 x 10	9 - 12	21	15	not reported
Schill & Landthaler[151]	1980	2 x 10	6 - 11	33	17	not reported
Traub & Thompson[152]	1981	2 x 10	6	32	16	9
Buvat et al.[154]	1983	2 x 10	4 - 12	25	25	10

and secondly by preventing the Leydig cell from maximally producing testosterone in response to LH (91,155-163). Vigersky and Glass (79) postulated that if estradiol formation was decreased by an aromatase inhibitor like Δ^1-testolactone (see fig. 2) than an improvement may occur in both sperm counts and fertility in patients with oligospermia.



Δ^1-Testolactone

Figure 2

Earlier Marynick et al. (164) reported that Δ^1-testolactone indeed produced a fall in serum estradiol levels in normal men with a concomitant rise in testosterone levels. From the paper of Vigersky and Glass (79) it became clear that Δ^1-testolactone administration for 6-12 months in a dose of 1 gr/day in 3 divided doses to 10 patients with idiopathic oligospermia, lowered serum estradiol level by 34%, increased testosterone, and androstenedione levels by 47 and 70% respectively and produced an increase in the T/E_2 ratio of 126%. Basal and LH-RH stimulated gonadotropin levels were uneffected by these changes. Nevertheless, sperm density almost doubled after 6-12 months of therapy and 3 men impregnated their wives. After this encouraging study only 2 further preliminary studies have appeared on the effect of Δ^1-testolactone on spermatogenesis, one by the same group using both Δ^1-testolactone and tamoxifen concomitantly and one study of Clark and Sherins (165,166). The later authors did not find any improvement at all after Δ^1-testolactone therapy in their patients with idiopathic oligospermia.

Aim of the study

In the section endocrine aspects of idiopathic oligospermia it was already stressed that in patients with idiopathic oligospermia Leydig cell function may also be impaired. A state of compensated Leydig cell function

function has been suggested in these patients, the reason of which is unknown so far.

Seminiferous tubular damage per se may lead to the secretion of some factor(s) blocking Leydig cell testosterone biosynthesis. In this context recent findings of Sharpe and other authors are of interest demonstrating the presence of an LH-RH-like peptide in rat testes (167170) and probably also in human testes (171-172). This peptide may modulate Leydig cell function. On the other hand, seminiferous tubular injury may lead to a secondary increase of LH (and/or LH-RH-like peptides) with subsequent desensitization of the Leydig cell and suppression of enzyme systems operative late in the Δ^4 pathway of testicular steroid biosynthesis, i.e., at the 17α-hydroxylase and 17,20-lyase step between progesterone and 17α-hydroxyprogesterone, and between 17α-hydroxyprogesterone and androstenedione respectively (fig. 3).

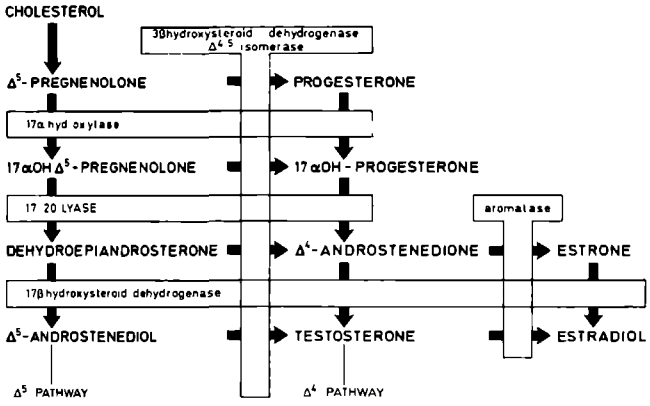


Figure 3: Steroidogenesis through the delta-4 and the delta-5 pathways in the testis

We, therefore hypothesized that if chronic Leydig cell desensitization were indeed present, stimulation by LH-RH should reveal an anomalous response of testosterone and its major precursor 17α-hydroxyprogesterone in patients with idiopathic oligospermia as compared with eusperm ic men.

In chapter II this hypothesis is tested.

As mentioned earlier, tamoxifen administration in doses varying from 20 to 30 mg daily has a beneficial effect on sperm density, total sperm counts and conception rates (145, 150-154). Preliminary data, however, suggested that this defect is dose dependent as Willis et al. (149) failed to demonstrate systemic changes in sperm parameters in oligospermic patients treated with the lower dosis 10 mg tamoxifen daily, despite clear-cut changes in serum gonadotropins and steroid levels reminiscent to those found after administration of the higher doses. These data prompted us to compare the effect of lower (5 and 10 mg once daily) and higher doses of tamoxifen (10 mg twice daily) on circulating hormone levels and sperm parameters in patients with moderate idiopathic oligospermia. The study was further designed to assess the predictary value of basal and LH-RH stimulated gonadotropin and steroid levels one month after starting therapy on the final outcome of spermatogenesis. Buvat et al. (153) provided preliminary evidence of a discriminating effect of tamoxifen induced hormonal changes soon after starting therapy on the eventual improvement of sperm parameters. In the case of amelioration of sperm, plasma testosterone vigorously increased whereas in the opposite case FSH levels overtly rose. Chapter III deals with the dose dependency and the value of hormonal data induced by tamoxifen administration in predicting the effect on sperm parameters.

Our finding of the presence of an intrinsic difference in the acute Leydig cell response to LH-RH between euspermic and oligospermic men, as reflected by the lack of an initial plasma testosterone rise and accumulation of 17-OHP over testosterone suggesting a 17,20-lyase block (160,163, 173-184) prompted us to search for a mechanism which could account for these differences. In rats and also in normal men ample evidence for a permissive role of estrogens has been adduced in the genesis of a 17,20-lyase block, which could be abolished by concomitant administration of an antiestrogen (185-186). In patients with idiopathic oligospermia an altered estrogen handling has been suggested (53,59,75,79,81,82). We therefore wondered whether sustained lowering of endogenous estrogen levels by chronic administration of the aromatase inhibitor Δ^1-testolactone may correct the anomalous Leydig cell response to LH-RH in these patients. (chapter IV).

Increased intratesticular estrogen concentrations or an inordinate

sensitivity of the germinal epithelium to their inhibiting effect have been postulated as the cause of the defective spermatogenesis in patients with idiopathic oligospermia (79). Evidence for a pivotal role of estrogens in the suppression of spermatogenesis was further adduced by Vigersky and Glass (79) who demonstrated that lowering estradiol levels by administration of the aromatase inhibitor Δ^1-testolactone (TL) for 6-12 months) indeed improves sperm density and fertility in patients with idiopathic oligospermia thus by a mechanism quite different from that of tamoxifen, which acts by competitive estradiol receptor blockade. In another preliminary study, Clark and Sherins (165), however, failed to demonstrate any effect at all of Δ^1-testolactone treatment on sperm parameters in their patients with idiopathic oligospermia. These conflicting data prompted us to monitor carefully the effect of sustained lowering of endogenous estrogen levels by Δ^1-testolactone administration for six months on pituitary-Leydig cell function and sperm parameters in patients with idiopathic oligospermia.

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DIFFERENTIAL EFFECT OF LUTEINIZING HORMONE-RELEASING HORMONE INFUSION ON TESTICULAR STEROIDS IN NORMAL MEN AND PATIENTS WITH IDIOPATHIC OLIGOSPERMIA

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ABSTRACT

Basal serum gonadotropin levels in 11 oligospermic men were significantly higher than in 9 euspermic control subjects, although most were still in the normal range. Basal serum testosterone (T), 17-hydroxyprogesterone (17-OHP) and estradiol (E_2) levels and their ratios did not differ significantly.

Continuous luteinizing hormone-releasing hormone (LH-RH) infusion (1 µg/minute for 180 minutes) during integrated blood sampling evoked similar gonadotropin responses in both groups but had a differential effect on T: in the control subjects T increased ($P < 0.01$) within 15 minutes to 1.5 times baseline, whereas in the oligospermic men T decreased ($P < 0.01$). From 60 minutes on, however, T also significantly rose in the oligospermic men, but the maximum increment was about half lower ($P < 0.01$) than in the euspermic men, despite virtually similar rises in 17-OHP. Only in the oligospermic men did the 17-OHP/T ratio increase ($P < 0.02$) during LH-RH, which is compatible with the occurrence of a 17,20-lyase block. Serum estradiol (E_2) did not increase in either group.

In conclusion, continuous LH-RH infusion uncovers an intrinsic difference in acute Leydig cell stimulation between euspermic and oligospermic men.

INTRODUCTION

Leydig cell function and reserve in idiopathic oligospermia have conflictingly been reported to be normal or impaired (1-4). On the strength of decreased plasma testosterone (T) levels associated with high normal or supranormal luteinizing hormone (LH) levels by some authors (1), a state of compensated Leydig-cell dysfunction has been suggested in these patients. The reason for this Leydig cell failure is not known. Seminiferous tubule damage per se may lead to the secretion of some factor(s) blocking Leydig cell T biosynthesis. In this context, the recent finding of Sharpe (5,6) is of interest, demonstrating the presence of an LH-releasing hormone (LH-RH)-like peptide in rat testes, probably secreted by the Sertoli cell and modulating Leydig cell T secretion. In man, also, LH-RH-like material immunologically differing from native hypothalamic LH-RH has been demonstrated in seminal plasma of healthy subjects as well as in patients with idiopathic oligospermia (7,8). Another explanation for the Leydig cell dysfunction in oligospermia might be that seminiferous tubule damage per se leads to a secondary increase of LH and/or an LH-RH-like peptide with subsequent desensitization of the Leydig cell, suppression of enzyme systems operating late in testicular steroid biosynthesis, i.e., at the 17,20-lyase and 17α-hydroxylase step, and hence reduced androgen secretion. Steinberger et al. (9) and Rodriguez-Rigau et al. (10) recently provided in vitro evidence of decreased 17,20-lyase activity in Leydig cells of at least some patients with idiopathic oligospermia and high normal serum gonadotropin levels. If chronic Leydig cell desensitization (11) were indeed present, low-dose LH-RH infusion should reveal an anomalous steroid response in patients with oligospermia, as compared with eugonadal euspermic men. The present study provides indirect evidence for an altered Leydig cell function in these patients.

MATERIALS AND METHODS

Nine healthy, potentially fertile volunteers [age, 30.3 $\pm$ 4.6 years standard deviation (SD)] ; mean sperm count, 41.5 $\pm$ 8 x 10^6/ml; abnormal forms < 40%; mean sperm motility graded 5 for rapidly and 1 for sluggishly progressive spermatozoa) (4-5), and 11 infertile patients with

idiopathic oligospermia (age, 31.8 ± 4.5 years [SD]; testicular volume, 19 ± 2 ml SD ; sperm count < 20 x 10^6/ml; mean, 12.1 ± 2.4 x 10^6/ml; abnormal forms, 67 ± 4%; mean sperm motility, 3) participated in this study after informed consent was obtained.

LH-RH (Relefact®, Hoechst Holland, Amsterdam, The Netherlands), 1 µg/minute, was administered by constant intravenous infusion (Harvard pump; Harvard apparatus; South Natick, MA) for 180 minutes to all participants.

Blood for LH, follicle-stimulating hormone (FSH), T, and estradiol (E_2) measurement was collected at 15-minute intervals by integrated blood sampling through a heparin-coated catheter (Kowarski catheter; Cormed, Middleport, N.Y.) using a constant withdrawal pump. The procedure started at t=-45 minutes at 4:00 P.M. (12). Serum LH and FSH levels were measured in all 15-minute samples, T and E_2 at t=0, 15, 30, 60, 120 and 180 minutes. Unfortunately, in only eight fertile and eight oligospermic men sufficient blood for additional 17-hydroxyprogesterone (17-OHP) measurement was available. Serum 17-OHP could only be measured in blood samples obtained at time intervals t=0, 30 and 120 minutes.

Serum LH and FSH levels were measured by a specific homologous double-antibody solid-phase radioimmunoassays (13) using antisera against highly purified human chorionic gonadotropin (hCG) and FSH without any significant mutual cross-reaction. The intraassay variability for duplicate measurements was 8.6% for LH and 8.1% for FSH. Normal ranges in euspermic men are, respectively, 4 to 15 mIU/ml and 0.9 to 4.5 mIU/ml. Serum E_2 levels were measured by specific radioimmunoassay using an antiserum raised against E_2-6-(O-carboxymethyl) oxime bovine serum albumin (intraassay coefficient of variation, 6.2%) (14). Serum concentrations of T (normal range at 8.00 A.M., 314 to 1337 ng/100 ml, mean ± SD: 568 ± 198 ng/100 ml) and 17-OHP (normal range at 8.00 A.M., 91 to 192 ng/100 ml, mean ± SD: 136 ± 39 ng/100 ml) were measured by radioimmunoassay after a paper chromatographic purification step using antisera raised in rabbits against 11-hydroxy-testosterone-11-hemisuccinate conjugated to albumin and in sheep against 11-desoxy-cortisol-21-hemisuccinate (16,17). The intraassay coefficients of variation are 6.1% and 4%, respectively. To avoid interassay variation, all samples from one individual were measured in the same assay. Statistical analysis was performed using Wilcoxon's paired rank test

(P values denoted by P), Wilcoxon's two-sample test (P*), Friedman's nonparametric analysis of variance (P**) and Spearman's rank correlation test (P***). Unless otherwise stated, the mean values ± 1 standard error of the mean (SEM) are given.

RESULTS

Basal serum gonadotropin and steroid levels

The mean basal serum LH level in the oligospermic men (13.7 ± 0.9 mIU/ml) was significantly higher ($P^* < 0.0001$) than in the eugonadal control subjects (7.0 ± 0.8 mIU/ml), although the individual levels were in the normal range in all but one patient (20 mIU/ml). The mean serum FSH level in the patients (3.3 ± 0.3 mIU/ml) also was significantly higher than in the healthy men (2.0 ± 0.2 IU/l, $P^* < 0.02$), but in nine of them the levels were still in the normal range. In the two remaining patients serum FSH levels were only slightly elevated, 4.7 and 5.3 mIU/ml, respectively. No statistically significant correlations were found between basal LH and FSH levels in either group (oligospermic men, $r = -0.03$; control subjects, $r = +0.30$; $P^{***} < 0.10$), or between FSH and the sperm count in the oligospermic patients ($r = 0.04$, $P^{***} < 0.10$).

The mean evening basal plasma T and 17-OHP levels in the oligospermic men (360 ± 33 ng/100 ml and 81 ± 11 ng/100 ml) were similar to those in the eugonadal men (321 ± 30 ng/100 ml and 82 ± 6 ng/100 ml, $P^* < 0.10$). The 17-OHP/T ratio in both groups also did not differ significantly (0.24 ± 0.03 versus 0.29 ± 0.02). The mean serum E_2 levels were similar in both groups of oligospermic and eugonadal men (41.9 ± 2.2 pg/ml versus 35.3 ± 2.7 pg/ml), and the E_2/T ratios also did not differ significantly from each other (0.012 ± 0.003 versus 0.013 ± 0.005).

Response to LH-RH infusion (figs. 1 to 3)

Serum LH levels significantly ($P < 0.01$) increased within 15 minutes after starting LH-RH infusion in both the eugonadal and oligospermic men, the increments not differing significantly between the two groups (ΔLH at t=15 minutes, 4.5 ± 1.6 mIU/ml versus 7.7 ± 2.6 mIU/ml).

After achieving peak levels at 45 minutes, LH levels plateaued. In contrast to literature data (17), in neither group was a biphasic LH response observed.

Serum FSH levels significantly increased ($P < 0.01$) within 15 minutes after starting the infusion in the healthy men (ΔFSH at t=15 minutes, 0.5 ± 0.2 mIU/ml) and within 30 minutes in the oligospermic patients (ΔFSH at t=30 minutes, 1.1 ± 0.4 mIU/ml). Although the increases of both LH and FSH were slightly better in the oligospermic group as compared with the control group, the difference in the mean areas under the LH (2077 ± 283 versus 1548 ± 233 area units) and FSH curves (205 ± 48 versus 124 ± 27 area units) lacked statistical significance.

In the control group, serum T levels increased without exception within 15 minutes to a mean level 1.5 ± 0.1 times baseline both at t=15 minutes (458 ± 33 ng/100 ml, $P < 0.01$ versus t=30 minutes (466 ± 29 ng/100 ml, $p < 0.01$ versus t=0). Thereafter, the mean level fell to a value not statistically different from the pretreatment value (385 ± 51 ng/100 ml) at t=60 minutes. A second rise to 458 ± 35 ng/100 ml (1.5 ± 0.2 times baseline, $P < 0.05$ versus t=0) was observed at 120 minutes after the start of the LH-RH infusion.

In contrast to the healthy control subjects, in the patients with idiopathic oligospermia serum T levels did not show an early increase despite similar LH increases, but decreased to a nadir level 329 ± 35 ng/100 ml (0.9 ± 0.05 times baseline) at t=15 minutes ($P < 0.01$ versus t=0) (fig. 1).

At t=30 minutes in eight out of ten patients tested, serum T levels were still equal to or below the baseline value.

The first statistically significant serum T increase was not observed until 60 minutes after starting the LH-RH infusion (398 ± 35 ng/100 ml, 1.1 ± 0.1 times baseline, $P < 0.02$ versus t=0). Thereafter, levels further increased to 1.3 ± 0.1 times baseline both at 120 and 180 minutes ($P < 0.01$ versus t=0). Comparing the increases in both groups, the mean maximum relative (76 ± 9% versus 37 ± 6%, $P^* < 0.01$) and absolute T increments (223 ± 14 ng/100 ml versus 119 ± 15 ng/100 ml, $P^* < 0.001$) were significantly higher in the control subjects than in the oligospermic men (fig. 2) despite similar mean maximum absolute increases in LH and FSH.

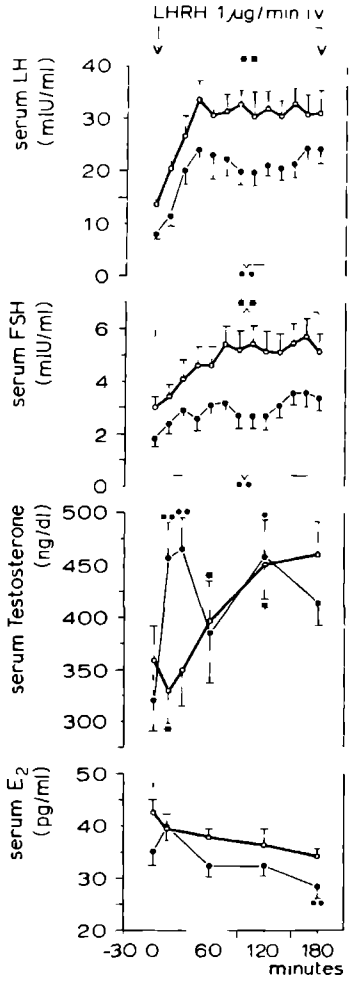

Figure 1: Mean (± SEM) serum LH, FSH, T and E_2 responses to constant LH-RH infusion (1 µg/minute for 180 minutes) in 9 (●--●) eugonadal men and 11 (o--o) patients with idiopathic oligospermia. The asterisks indicate statistically significant changes as compared with the basal value. *P < 0.05. **P < 0.01.

In contrast to serum T, in the control group serum 17-OHP levels remained unchanged during the 30 minutes after starting LH-RH infusion (88 ± 6 ng/100 ml). Thereafter, the levels increased to 1.8 ± 0.2 times baseline at 120 minutes (139 ± 11 ng/100 ml, P < 0.05 versus t=0).

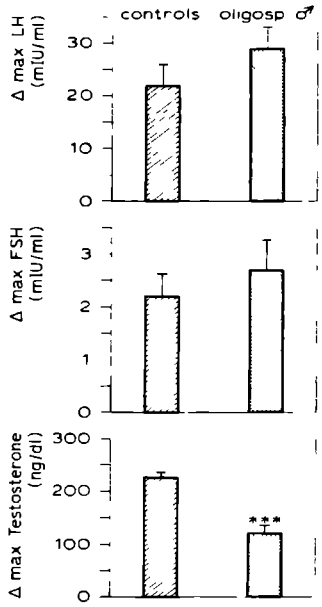

Figure 2: Mean maximum absolute serum LH, FSH and T increments (± SEM) in response to constant LH-RH infusion (1 μg/minute for 180 minutes) in 9 eugonadal men and 11 patients with idiopathic oligospermia. The asterisks indicate a statistically significant difference ($P < 0.001$) between both groups.

In the oligospermic patients, 17-OHP levels at 30 minutes also did not differ significantly from the pretreatment level (75 ± 8 ng/100 ml), but thereafter, levels significantly increased to 138 ± 13 ng/100 ml, 2.0 ± 0.3 times baseline at t=120 minutes ($P < 0.01$ versus t=0). serum T levels significantly rose in the first 30 minutes and 17-OHP levels remained virtually unchanged (Fig.3), the 17-OHP/T ratio in the healthy control subjects fell (-0.09 ± 0.03) and 0.7 ± 0.1 times baseline ($P < 0.05$ versus $t = 0$) and thereafter rose to pretreatment levels at t=120 minutes (1.0 ± 0.1 times baseline, $P < 0.10$ versus t=0). In the oligospermic groups the 17-OHP/T ratio virtually remained unchanged (-0.01 ± 0.03, $P^* < 0.10$ versus healthy men) until 30 minutes and then significantly increased to 1.5 ± 0.2 times baseline ($P < 0.02$ versus t=0) at t=120 minutes. As the mean rises of serum 17-OHP at 120 minutes were identical in both groups of healthy men and oligospermic patients

(57 ± 14 ng/100 ml versus 57 ± 13 ng/100 ml), but the T increments were significantly lower in the latter (91 ± 19 ng/100 ml versus 189 ± 33 ng/100 ml), the mean increase in the 17-OHP/T ratio in the oligospermic men was significantly higher than in the control subjects (0.090 ± 0.03 versus 0.002 ± 0.04, P* < 0.02) (fig. 3).

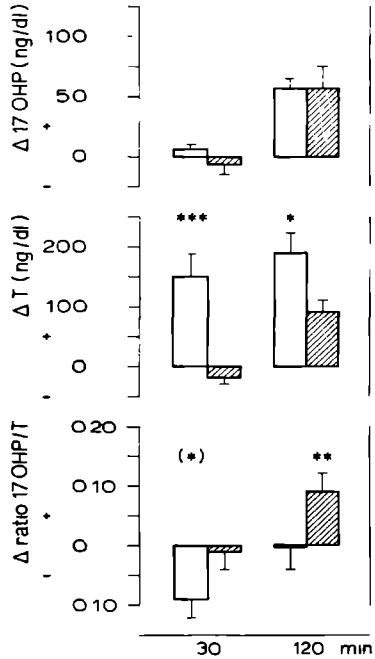

Figure 3: Mean serum 17-OHP, T, and 17-OHP/T ratio increments (± SEM) in response to constant LH-RH infusion (1 µg/minute for 180 minutes) at 30 and 120 minutes in 8 eugonadal men ☐ and 8 patients with idiopathic oligospermia ▨. The asterisks indicate statistically significant differences between the two groups. (*)P < 0.10. *P < 0.05. **P < 0.02. ***P < 0.002.

In contrast to T, in the control group serum E_2 levels did not change significantly between 0 and 15 minutes, but thereafter the levels significantly (P** < 0.0025) decreased to lowest values 29 ± 2 pg/ml, at 180 minutes (P < 0.01 versus t=0). In the oligospermic men,

E_2 levels at t=15 were also similar to the baseline value. Thereafter, the levels showed a downward trend to lowest values, 34 $\pm$ 1 pg/ml, at t=180 minutes (P** < 0.10). At t=180 minutes, the mean E_2 levels in the oligospermic men were significantly higher than in the healthy control subjects (P < 0.05).

DISCUSSION

The mean basal gonadotropin levels in the patients with severe to moderate oligospermia from the present study were significantly higher than in the control subjects despite normogonadotropism in most of the patients. Basal sex steroid levels (T, 17-OHP and E_2), however, were similar to those in the healthy control subjects, suggesting the presence of compensated Leydig cell failure in these oligospermic men (1). Low-dose continuous LH-RH infusion and integrated blood sampling, however, disclosed differential response patterns of serum T as well as 17-OHP between the control subjects and the oligospermic patients.
The absolute and relative maximum increments of serum T in response to the LH-RH infusion in the oligospermic men were only half the increase observed in the eugonadal control subjects despite comparable or even higher rises in serum gonadotropin levels in the former. Schwarzstein et al. (3) came to the same conclusion comparing the effect of a rapid bolus injection of 50 µg LH-RH in healthy men and normogonadotropic patients with oligospermia. These ivestigators also found significantly lower rises of serum T in the latter, 30 minutes after LH-RH loading. In contrast, Massala et al.(4) did not observe any difference in the response of T or E_2 after a similar high-dose bolus injection. The present data and Schwarzstein's data on a differential T response after short-term LH-RH infusion or a bolus injection confirm data on long-term Leydig cell stimulation by hCG in oligospermic men (1). A subnormal rise of serum T was observed after 4 to 5 days of hCG stimulation in patients with seminiferous tubule pathology, suggesting that the pathologic process also involves the intertubular compartment of the testis (1).

In this context it is of particular interest that despite significantly lower T increases in response to LH-RH infusion in the oligospermic men, the increments of its major precursor 17-OHP at 120 min-

utes after starting the infusion were almost identical. Therefore, in contrast to the findings in healthy control subjects, in whom 17-OHP and T ran a parallel course, leaving the 17-OHP/T ratio unchanged, in the oligospermic patients there was a dissociated course, the 17-OHP/T ratio increasing by about 50%. Although it is precarious to translate changes in circulating steroid levels into intratesticular changes, the relative accumulation of 17-OHP over T during LH-RH stimulation in the oligospermic men tentatively might be interpreted to mean the occurrence of an enzymatic impairment localized at the 17,20-lyase step (18), assuming that no alternate pathway for the precursor is available and other enzymes or factors pertaining to hormone secretion remain unaltered.

It has to be noted, however, that in the unstimulated basal condition there was no relative accumulation of the T precursor and therefore no evidence of an enzyme defect. These data are reminiscent of similar findings in Klinefelter's syndrome (16), and in patients with varicocele in whom only gonadotropin stimulation uncovered the presence of a putative enzymatic impairment (19). In contrast, Steinberger et al.(9) and Rodriguez-Rigau et al.(10) found in vitro evidence of accumulation of 17-OHP over T in the testes of some patients with idiopathic oligospermia both under basal and stimulated conditions.

Continuous LH-RH infusion in addition to the differences in T and 17-OHP responses revealed another hitherto unreported dissociation, namely, in the acute response of T soon after starting the infusion. In the healthy control subjects, serum T levels showed a biphasic response with an early peak at 15 and 30 minutes after starting the infusion. In contrast, in the oligospermic men, serum T levels did not show the early peak but rather a fall at 15 minutes in all but one patient. A statistically significant rise in serum T levels was not observed until 60 minutes after starting the LH-RH infusion, but the increases remained lower than in the control subjects. It has to be noted that in the healthy control subjects serum T levels showed the early increase without concomitant change in 17-OHP levels, suggesting the presence of a readily releasable T pool which was apparently absent in the oligospermic men. The early T rise occurring after infusion of only 15 µg of LH-RH confirms data of Schwarzstein et al.(20,21), who found maximum T levels 16 minutes after a 50 µg intravenous bolus injection of LH-RH in

normal men. As in our series, some subjects showed a clear early T response with virtually unchanged LH levels. These data contrast, however, with the data of Nankin et al., who failed to demonstrate an early T rise not only after a subcutaneous injection of 100 µg LH-RH but also after an intravenous bolus injection (22) or infusion of LH (23) leading to three- to ninefold increases of circulating LH levels within 5 to 30 minutes. The slower route of LH-RH administration might account for the divergent finding in Nankin's study. The lack of an early T rise in the oligospermic patients from the present study contrasts with the findings in the healthy men and could not be presaged from the basal sex steroid levels, which were similar in both groups. The only difference between the healthy and oligospermic men was the twice higher basal LH level in the latter. This chronic hypergonadotropism could have led to depletion of the rapidly releasable T store, thereby preventing the early T response induced by LH-RH itself or the LH-RH-mediated LH increase. An alternative explanation for the lack of an acute LH-RH-induced T response may be that in idiopathic oligospermia seminiferous tubule damage leads to increased gonadotropin levels and local secretion of LH-RH-like peptides which eventually inhibit Leydig cell steroidogenesis.

Schwarzstein et al.(3), however, administering 50 µg LH-RH as an intravenous bolus injection at 8.00 A.M., also in patients with idiopathic oligospermia, found an albeit subnormal, but still statistically significant, early T increase 30 and 45 minutes after the injection. Although diurnal variations in the T response to LH-RH should be taken into consideration - the LH-RH infusion tests in the present study were performed in the evening - they cannot explain the lack of an initial T increase in the oligospermic men, because such an increase did occur in our healthy subjects and Schwarzstein's (3). Differences in the method of LH-RH administration could account for the aberrant T response, which may only become overt after slow low-dose LH-RH infusion and may be obscured by massive LH-RH bolus injections.

Nevertheless, the data in this study illustrate that in idiopathic oligospermia Leydig cell function is anomalous. Chronic desensitization by elevated gonadotropins and/or locally secreted LH-RH-like peptides due to seminiferous tubule damage, leading to enzymatic impairment, may be one of the numerous causes of the Leydig cell dysfunction. Further-

more, low-dose LH-RH infusion uncovers an intrinsic difference in acute Leydig cell stimulation between healthy men and patients with idiopathic oligospermia with an early serum T rise in the former but a fall in the latter.

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EFFECT OF LOWER VERSUS HIGHER DOSES OF TAMOXIFEN ON PITUITARY-GONADAL FUNCTION AND SPERM INDICES IN OLIGOZOOSPERMIC MEN

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SUMMARY

Administration of the antiestrogen tamoxifen for one month to 12 patients with idiopathic oligozoospermia significantly increased the mean basal testosterone (T) level and the responses of luteinizing hormone (LH) and follicle stimulating hormone (FSH) to constant luteinizing hormone-releasing hormone (LH-RH) infusion but did not significantly influence the mean estradiol (E_2) levels or the E_2 over testosterone ratio. Mean sperm concentration and total sperm output increased by about 70% after a mean treatment period of 5.5 $\pm$ 0.4 months. No statistically significant difference was found between the two subgroups of patients treated with either the lower (5 or 10 mg once daily) or higher dose of tamoxifen (10 mg twice daily) with respect to basal or LH-RH stimulated gonadotropin and testosterone response or the E_2/T ratio and the effect on sperm density and total sperm output. In both subgroups the sperm motility and morphology remained unchanged.

In conclusion higher doses of tamoxifen in this study prove not to be superior to lower doses in improving mean sperm density and total sperm output. The relative small percentage of patients achieving normalization of only these sperm parameters pleads for further search for more effective selection of patients and other more effective treatment modalities in patients with idiopathic oligozoospermia.

INTRODUCTION

Since Comhaire (1976) first reported on the use of the competitive non-steroidal anti-estrogen tamoxifen in patients with idiopathic oligozoospermia, ample reports (Willis, et al. 1977; Vermeulen and Comhaire 1978; Schill and Landthaler 1980; Traub and Thompson 1981; Bartsch and Schreiber 1981; Buvat, et al. 1982; Frajese, et al. 1983) have appeared dealing with its beneficial effect on sperm density and total sperm counts. Preliminary data suggested that this effect is dose dependent as Willis, et al. (1977) failed to demonstrate systematic changes in sperm parameters in oligozoospermic patients treated with 10 mg tamoxifen daily in stead of the 20 mg used by Comhaire (1976).

However, scrutinizing the scarce literature data on hormonal effects of higher and lower doses of tamoxifen, no overt difference was found in hormonal parameters between these studies (Comhaire 1976; Willis, et al. 1977; Vermeulen and Comhaire 1978). These data prompted us to compare the effect of lower (5 and 10 mg once daily) and higher doses (10 mg twice daily) of tamoxifen on circulating hormone levels and sperm parameters in patients with idiopathic oligozoospermia. The study was further designed to assess the predictary value - if any - of basal and LH-RH stimulated gonadotropin and steroid levels on spermatogenesis one month after starting tamoxifen therapy. Fabian, et al. (1980) namely showed that steadystate levels of tamoxifen are achieved after 4 weeks of therapy the levels remaining constant for periods up to 6 months thereafter.

MATERIALS AND METHODS

Fifteen normogonadotropic patients with idiopathic oligozoospermia and longstanding infertility initially entered the study after giving informed consent and after approval of the protocol by the hospital ethical committee. Only 12 of them completed the study (age, 32.9 $\pm$ 5.1 SD years). All of these patients had a normal testicular volume assessed with a Prader orchidometer (volume: 20 $\pm$ 1.7 ml SD). There was no history of inflammatory testicular disease and none of the patients had previously received any form of hormonal treatment. The presence of varicocele was excluded in all of them. Their wives showed normal ovu-

latory cycles and patent tubes on hysterosalpingography, but despite good mucus quality negative post coital tests. The sperm density determined at 1 month intervals in at least 2 specimens of semen freshly obtained by masturbation after sexual abstinence for 4 to 5 days was 9.2 $\pm$ 1.9 SE x 10^6/ml. The mean total sperm output per ejaculate was 38.3 $\pm$ 8.3 x 10^6/ml (table II). Mean sperm motility (graded 5 for rapidly progressive and 1 for sluggishly progressive spermatozoa) was 3 and the mean percentage abnormal forms 66 $\pm$ 3.5 SE %.

Of the twelve patients completing the study - five initially entered in each dose group - seven received the lower dosis of 5 mg (n=3) or 10 mg (n=4) of tamoxifen (Nolvadex®, ICI Ltd) once daily - these patients were further denoted as the "lower dose group" - and five received the higher dose of 10 mg twice daily ("higher dose group").

The duration of treatment was six months. Before and after one month of tamoxifen therapy responsiveness of LH, FSH, T and E_2 to LH-RH (Relefact®, Hoechst Holland, Amsterdam, The Netherlands) 1 µg/minute administered by constant intravenous infusion (Harvard pump; Harvard apparatus; South Natrick, MA) for 180 minutes was assessed (Fauser et al. 1983; Dony et al. 1984). Blood for LH, FSH, T and E_2 measurement was collected at 15 minute intervals by integrated blood sampling through a heparin coated catheter (Kowarski-catheter, Cormed, Middleport, N.Y.) using a constant withdrawal pump.

The procedure started at t=-45 minutes at 4.00 P.M. Serum LH and FSH were measured in all 15 minute samples, T and E_2 in the samples obtained at t=0,15,30,45,60,90,120,150 and 180 minutes. Serum LH and FSH levels were measured by two specific homologous double-antibody solid-phase radioimmunoassays (DASP) (van Geelen, et al. 1981), using antisera against highly purified human chorion gonadotropin (hCG) and FSH without any significant mutual cross reaction.

The intraassay variability for duplicate measurements was 8.6% for LH and 8.1% for FSH. Normal ranges in eupermic men are 4 - 15 and 0.9 - 4.5 mIU/ml respectively (Dony, et al. 1984).

Serum E_2 levels were measured by a specific radioimmunoassay (RIA) using an antiserum raised against E_2-6-(O-carboxymethyl) oxime bovine serum albumin (intraassay coefficient of variation 6.2%) (Thomas, et al. 1977). Serum concentration of T was measured by RIA after a paper chromatographic purification step using an antiserum raised in rabbits

against 11-hydroxy-testosterone-11-hemisuccinate conjugated to albumin (intraassay coefficient of variation 6.1%) (Smals, et al. 1976). To avoid interassay variation samples from one individual were measured in the same assay as much as possible.

Statistical analysis was performed using Wilcoxon's paired rank test (P values denoted by P) and Wilcoxon's two sample test (P*). Unless otherwise stated the mean values $\pm$ 1 SEM are given.

RESULTS

Effect of LH-RH infusion on serum gonadotropin and steroid levels before tamoxifen administration (fig. 1).

The mean basal LH and FSH levels in the twelve oligozoospermic men 13.4 $\pm$ 0.8 mIU/ml and 3.0 $\pm$ 0.4 mIU/ml, respectively (table I) were in the normal range though significantly higher than the values reported earlier for eugonadal control subjects (LH, 7.0 $\pm$ 0.8 mIU/ml, $P^* < 0.01$ versus oligozoospermic men, FSH, 2.0 $\pm$ 0.2 mIU/ml, $P^* < 0.02$) (Dony et al. 1984). In only three of the patients serum LH levels (16, 16 and 20 mIU/ml) and in two serum FSH levels (4.7 and 5.3 mIU/ml) were slightly elevated. Serum T was in the normal range in all patients, whereas serum E_2 levels and the E_2/T ratio were of the same order of magnitude as reported earlier for normal men (35.3 $\pm$ 2.7 pg/ml and 0.013 $\pm$ 0.005, respectively) (Dony, et al. 1984). No statistically significant differences were found between the two groups of patients to be treated with the lower or higher doses of tamoxifen in any of the basal or stimulated pituitary-gonadal functions or sperm indices (table I and II).

Considering the group of twelve oligozoospermic patients as a whole, before tamoxifen therapy serum T levels significantly ($P < 0.05$) fell within 15 minutes after starting LH-RH infusion. The first statistically significant T increase was observed after 60 minutes ($P < 0.05$ versus t=0) and peak levels were achieved after 90 minutes (1.3 $\pm$ 0.1 times baseline, $P < 0.01$ versus t=0). Thereafter, the mean T levels plateaued and remained significantly elevated until the end of the test.

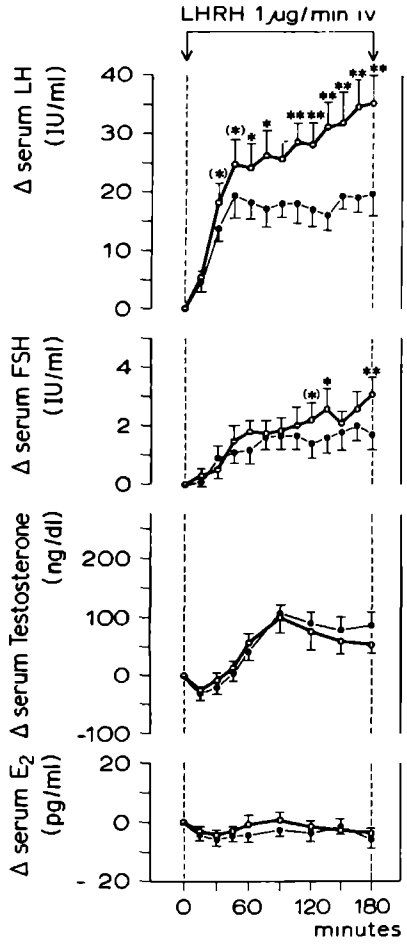

Figure 1: Mean (± SEM) serum LH, FSH, T and E_2 increments in response to constant LH-RH infusion (1 µg/minute for 180 minutes) in 12 patients with idiopathic oligozoospermia before (●--●) and after (o--o) 1 month of tamoxifen treatment. The asterisks indicate statistically significant differences between both groups (*) $0.05 < P < 0.10$. *$P < 0.05$. **$P < 0.01$.

Comparing the two groups to be treated with the lower and higher dose of tamoxifen no statistical significant differences were found at any point of the curves between the absolute T levels (data not shown) or the maximal T increments (table I).

Table I: Basal and LH-RH (1 µg/minute for 180 minutes) stimulated serum LH, FSH, T and E_2 levels and the ratio E_2 over T (mean ± SEM) in 12 patients with idiopathic oligozoospermia before and after treatment with lower (5 or 10 mg Nolvadex® daily, n=7) or higher (10 mg twice daily, n=5) doses of tamoxifen (TAM) for one month

	all patients (n = 12)		low dose group (n = 7)		high dose group (n = 5)	
	before TAM	after 1 month of TAM	before TAM	after 1 month of TAM	before TAM	after 1 month of TAM
basal LH (mIU/ml)	13.4 ± 0.8	15.2 ± 1.2	13.4 ± 1.3	13.2 ± 1.0	13.6 ± 1.0[a]	17.8 ± 1.9[b]
max LH (mIU/ml)	27.2 ± 3.7	38 ± 5**	27.6 ± 5.8	34.3 ± 5.1*	26.8 ± 4.3[a]	43.0 ± 9.9[b](*)
area LH (area units)	201 ± 26	312 ± 43**	202 ± 32	298 ± 54*	200 ± 45[a]	332 ± 71[b](*)
basal FSH (mIU/ml)	3.0 ± 0.4	3.5 ± 0.5	3.2 ± 0.4	3.4 ± 0.6	2.7 ± 0.6[a]	3.6 ± 0.8[b]
max FSH (mIU/ml)	2.5 ± 0.5	3.5 ± 0.7**	2.9 ± 0.8	4.2 ± 0.9(*)	1.8 ± 0.5[a]	2.6 ± 0.9[b]
area FSH (area units)	9.3 ± 3.2	11.8 ± 3.4	11.0 ± 4.9	13.9 ± 5.3	7.0 ± 3.1[a]	9.0 ± 3.7[b]
basal T (ng/100 ml)	406 ± 47	517 ± 43*	383 ± 80	501 ± 45	437 ± 57[a]	540 ± 82[b]
max T (ng/100 ml)	126 ± 27	127 ± 27	145 ± 18	132 ± 41	114 ± 16[a]	120 ± 31[b]
basal E_2 (pg/ml)	41 ± 3	48 ± 4	44 ± 3	43 ± 3	38 ± 5[a]	56 ± 8[b]
basal E_2/T (ratio)	0.012 ± 0.002	0.010 ± 0.001	0.014 ± 0.002	0.009 ± 0.001	0.010 ± 0.001[a]	0.011 ± 0.001[b]

(*) $0.05 < P < 0.10$ vs before tamoxifen
* $P < 0.05$ vs before tamoxifen
** $P < 0.01$ vs before tamoxifen

a $P > 0.10$ vs before low dose tamoxifen
b $P > 0.10$ vs after low dose tamoxifen

In contrast to T, serum E_2 levels virtually remained unchanged throughout the LH-RH test both when considering the group as a whole (fig. 1) and the two subgroups (data not shown).

Effect of tamoxifen administration irrespective of the dose on LH-RH stimulated gonadotropin and steroid levels and serum output in the oligozoospermic patients (fig. 1).

Considering the group of twelve oligozoospermic patients as a whole, one month of tamoxifen administration did not significantly increase the mean basal serum LH or FSH levels (fig. 1, table I). The mean serum T values, however, significantly increased ($P < 0.05$). In contrast, serum E_2 levels virtually remained unchanged.

One month of tamoxifen treatment without exception enhanced the LH response to LH-RH infusion, the maximum LH increments and the area under the LH curve (fig 1, table I). At almost all time intervals from 60 minutes on the LH increments were significantly higher ($P < 0.05$ to $P < 0.01$) during tamoxifen therapy. Tamoxifen also enhanced the maximum FSH response to LH-RH but did not influence the area under the curves (table I). The T increments in response to LH-RH during tamoxifen therapy were similar to those observed without tamoxifen at all time intervals (fig. 2). The mean serum E_2 levels virtually remained unchanged during LH-RH infusion both before and during tamoxifen administration. Although the absolute levels were somewhat higher in the latter condition, the E_2 changes did not differ significantly (fig. 1).

The mean sperm density and total sperm counts significantly increased ($P < 0.05$) during tamoxifen therapy from 9.2 ± 1.9 before to $16.3 \pm 3.7 \times 10^6$/ml and 38.3 ± 8.3 to $64.1 \pm 10.8 \times 10^6$, respectively, 5.5 ± 0.4 months after starting treatment (table II). Sperm motility and the percentage abnormal forms, however, did not improve significantly. It has to be noted that no statistically significant changes were observed during the first two months of treatment. Only one pregnancy occurred during the treatment period, i.e. in the 2 x 10 mg tamoxifen group.

Table II: semen parameters (mean ± SEM) in 12 patients with idiopathic oligozoospermia before and during treatment with tamoxifen, 5 or 10 mg nolvadex®, daily (low-dose, n = 7) or 20 mg (high-dose, n = 5) for a mean period of 5.5 ± 0.4 months

	dose of tamoxifen (mg)	before tamoxifen (months) -2	-1	mean (-2/-1)	during tamoxifen (months) +1	+2	>3 (5.5±0.4)
Sperm density (10^6/ml)	low	9.8 ± 2.5	12.9 ± 2.5	11.4 ± 2.6	12.2 ± 4.6	12.7 ± 5.5	18.1 ± 4.8
	high	5.4 ± 1.4	6.6 ± 2.3	6.0 ± 1.7	11.6 ± 5.5	-	13.9 ± 5.8
	all patients	8.0 ± 1.7	10.3 ± 2.4	9.2 ± 1.9	11.9 ± 3.5	10.4 ± 4.1	16.3 ± 3.7*
Total sperm count (10^6/ml)	low	37.5 ± 13.4	47 ± 12.3	42.2 ± 9.9	37.9 ± 20	44.8 ± 13.4	69.2 ± 13.1*
	high	27.1 ± 10.0	38.0 ± 18.0	32.6 ± 13.8	38.5 ± 15.3	-	57.1 ± 17.8
	all patients	33.3 ± 9.0	43.3 ± 10.0	38.3 ± 8.3	38.2 ± 21	41.5 ± 11.7	64.1 ± 10.8*
percentage abnormal forms (%)	low	64 ± 4	68 ± 4	66 ± 4	72 ± 4	65 ± 3	66 ± 6
	high	68 ± 7	65 ± 6	67 ± 6	68 ± 8	-	69 ± 6
	all patients	66 ± 4	67 ± 3	66 ± 3	70 ± 4	68 ± 4	67 ± 4

*P < 0.05 vs before tamoxifen

Effect of the lower and higher dose of tamoxifen on basal and LH-RH stimulated gonadotropin and steroid levels and sperm parameters (fig. 2, table I and II)

No statistically significant difference was found in the mean basal serum LH, FSH, T or E_2 levels one months after starting tamoxifen treatment between the lower and higher dose groups. The mean E_2/T ratio was also similar (table I). LH-RH administration one month after starting the lower or higher dose tamoxifen treatment similarly increased LH and FSH levels in both groups (fig. 2).
At no time point of the curves a statistically significant difference could be demonstrated in the LH or FSH increments above baseline (fig. 2) or in the mean maximum LH and FSH increments and the areas under the curves (table I). The LH-RH induced mean T increases and also did not differ significantly between the two groups at any point of the curves nor did the maximum T increments (table I).

The only statistically significant difference observed between the lower and higher dose group was in the LH-RH induced E_2 increments which were significantly higher in the higher dose group (fig. 2) at several time intervals after starting the infusion ($P^* < 0.05$).

Before starting tamoxifen therapy no statistically significant difference was found in the sperm indices between the lower and higher dose tamoxifen groups (table II). Tamoxifen administration for a mean duration of 5.5 ± 0.4 months increased sperm density by $6.8 \pm 2.6 \times 10^6$/ml in the lower dose and by $7.8 \pm 6.4 \times 10^6$/ml ($P^* < 0.10$ versus lower dose) in the higher dose group. Normal sperm density ($> 40 \times 10^6$) was achieved in only one patient of either group. Sperm motility and the percentage abnormal forms were unchanged in both groups. In 6 out of 7 patients of the lower dose and 4 out of 5 of the higher dose group total sperm counts increased, whereas levels above 100×10^6 were achieved in respectively 2 and 1 of them. It has to be emphasized that both subsets within the lower tamoxifen group behaved quite similarly in their gonadotropin and steroid response to LH-RH administration and their changes in sperm parameters.

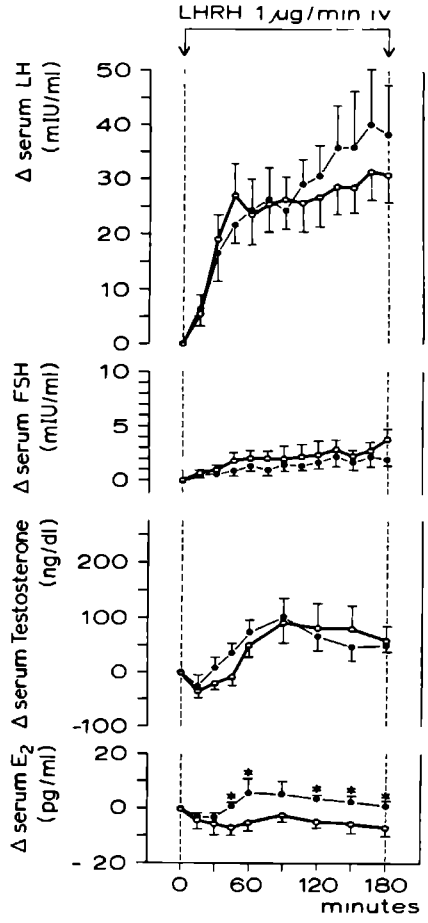

Figure 2: Mean (± SEM) LH, FSH, T and E_2 increments in response to constant LH-RH infusion (1 µg/minute for 180 minutes) in patients with idiopathic oligozoospermia after 1 month of tamoxifen therapy, 7 on the lower dose of 5 or 10 mg (o--o) and 5 on the higher dose of 20 mg (●--●). The asterisks indicate statistically significant differences between both groups, *P < 0.05.

DISCUSSION

The present study is in agreement with those of most authors reporting some beneficial effect of tamoxifen on mean sperm density and mean

total sperm count, but not on the progressive motility or sperm morphology in patients with idiopathic oligozoospermia (Comhaire 1976; Vermeulen and Comhaire 1978; Frajese et al. 1983). Although the increase was poor in absolute terms, the mean sperm density and total sperm count in the patients from the present study improved by about 70% after a mean treatment period of about 1/2 year (range 3 to 9 months), whereas in 2 respectively 3 of the patients these indices normalized. Similar increments in sperm density (66-182%) were reported by other authors (Comhaire 1976; Schill and Landthaler 1981; Traub and Thompson 1981; Buvat, et al. 1982; Frajese, et al. 1983) using the higher dose of 20 or even 30 mg of tamoxifen daily for the same period of time.

In contrast to Willis et al. (1977), who failed to demonstrate statistically significant effect of the lower dose of 10 mg tamoxifen on sperm density in 9 patients with idiopathic oligozoospermia treated for 6 months, 6 out of 7 patients from the present study treated with the same or even lower dose of 5 mg tamoxifen showed an increase of the sperm density and/or total sperm count similar to that found in the patients treated with the higher dose. Although a placebo effect can not be excluded (Willis, et al. 1977) the data in the present study favour the conclusion that lower doses of tamoxifen (5 and 10 mg) are equally, although modestly, effective in raising sperm densities and sperm counts as the commonly used higher doses.

Taking into account the earlier mentioned pharmacokinetics of tamoxifen resulting in steady-state blood levels from one month of therapy on, the concordance in therapeutic effect was predictable in the light of the similarity in basal and LH-RH stimulated gonadotropin and steroid responses in the lower and higher dose therapy group. Only serum E_2 levels were slightly significantly higher during LH-RH infusion in the latter group. As high E_2 levels have been reported to inhibit spermatogenesis directly (Steinberger 1975; Kalla, et al. 1980) or indirectly (Nozu, et al. 1982; Smals, et al. 1980), the higher E_2 levels in this group would rather militate against the use of the higher dose. As mentioned above the changes in the semen parameters during tamoxifen therapy in the present study were not associated with statistically significant changes in basal gonadotropin levels confirming recent data of Buvat et al. (1982) and Frajese et al. (1983), but

in contrast with reports of other authors (Willis, et al. 1977; Buvat, et al. 1982).

Enhanced sensitivity of LH, and FSH to LH-RH administration and during tamoxifen treatment (Willis, et al. 1977; Vermeulen and Comhaire 1978; Gooren 1981) arise in basal serum T levels (Willis, et al. 1977; Vermeulen and Comhaire 1978; Buvat, et al. 1982; Frajese, et al. 1983; Gooren 1981) both may account for the improvement in sperm density. It has to be emphasized that this improvement during antiestrogen treatment occurred despite lack of statistically significant changes in the E_2/T ratio (present study) or even despite increases in this ratio (Vermeulen and Comhaire 1978). Although the precise mechanism by which tamoxifen improves spermatogenesis still remains to be elucidated, the present study demonstrates that the lower and higher doses - all probably were on the plateau of the dose response curve - were equally effective in increasing mean sperm density and total sperm counts in patients with idiopathic oligozoospermia.

Nevertheless, the relatively small percentage of patients showing - quantitative - normalization of their sperm output (16% in the present study and about 25-30% in literature) pleads for further search for more effective selection of patients and more effective treatment modalities in these patients e.g. use of more specific antiestrogens (Vigersky, et al. 1981). Studies are now in progress on the effect of the aromatase enzyme inhibitor testolactone on sperm indices in patients with idiopathic oligozoospermia.

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EFFECT OF AROMATASE INHIBITION BY Δ^1-TESTOLACTONE ON BASAL AND LUTEINIZING HORMONE-RELEASING HORMONE STIMULATED PITUITARY AND GONADAL HORMONAL FUNCTION IN OLIGOSPERMIC MEN

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ABSTRACT

Aromatase inhibition by Δ^1-testolactone (TL), 500 mg twice daily for 4 weeks, in 9 patients with idiopathic oligospermia lowered circulating estradiol (E_2) levels by about 30%, enhanced the secretion of follicle stimulating hormone (FSH) (+ 30%), 17-hydroxyprogesterone (17-OHP) (+ 40%) and testosterone (T) (+ 30%), but did not affect serum luteinizing hormone (LH) levels. Despite E_2 lowering, there was an accumulation of 17-OHP over T suggesting 17,20-lyase inhibition. Unexpectedly, administration of TL almost completely deleted the T response to continuous luteinizing hormone-releasing hormone (LH-RH) infusion present before TL therapy, despite similar gonadotropin release. Because the 17-OHP response to the LH-RH infusion was even higher during therapy, the 17,20-lyase lesion seemed aggravated despite substantial reduction of E_2 levels.

Although the present data suggest that estrogens play a less dominant role in the origin of the late steroidogenetic lesion than previously assumed, the suggestion also arises that TL per se, in addition to its antiestrogenic action, exerts an inhibiting effect on the 17,20-lyase locus, which may obscure the beneficial effect of reducing E_2.

INTRODUCTION

Δ^1-Testolactone (TL), 17α-oxa-D-homo-1,4 androstadiene-3,17-dione, a testosterone (T) derivative without major intrinsic androgenic activity, is a potent competitive inhibitor of aromatase activity and blocks the peripheral aromatization of androgens to estrogens (1-5), at least in men (6).

In patients with idiopathic oligospermia an increase in testicular intratubular estrogen concentration and/or an inordinate sensitivity of the germinal epithelium to its inhibitory effect has been postulated (3). Vigersky and Glass (3) adduced preliminary evidence that decreasing estradiol (E_2) formation by the administration of TL may indeed improve sperm counts and fertility in these patients, although the data have been challenged by others (7). Apart from an altered estrogen handling, in vitro and in vivo evidence point to the presence of a 17,20-lyase block in idiopathic oligospermia (8-10).

Recently we demonstrated an intrinsic difference in the acute Leydig cell response between euspermic and oligospermic men because the latter failed to show the initial rise in plasma T during continuous luteinizing hormone-releasing hormone (LH-RH) infusion present in healthy controls (10). Furthermore they exhibited a blunted and late rise of T but, in contrast, a normal increase in 17-hydroxyprogesterone (17-OHP), which is compatible with the presence of a block late in the steroid biosynthesis at the locus of the 17,20-lyase enzyme. In rats and also in healthy men a permissive role of estrogens - exogenous or endogenous, as provoked by human chorionic gonadotropin (hCG) administration - has been demonstrated in the genesis of such lesion (11-13). This block could be abolished by the concomitant administration of antiestrogens (11,14,15).

The present study therefore was designed to investigate whether sustained lowering of endogenous estrogen levels by chronic administration of TL might influence the anomalous Leydig cell response to LH-RH in patients with idiopathic oligospermia.

MATERIALS AND METHODS

Nine normogonadotropic oligospermic men, [30.6 $\pm$ 3.9 years of age

(standard deviation)], with proven infertility of at least one year's duration were intensively studied during the first four weeks of their long-term treatment with TL after informed consent was obtained. Their sperm density was less than 20 x 10^6/ml, the mean sperm count was 8.1 $\pm$ 1.3 x 10^6/ml, abnormal forms were 66% $\pm$ 3%, the mean sperm motility 3 (motility graded 5 for rapid progression and 1 for immobile sperm) and all had normal testicular volumes (19 $\pm$ 2 ml). Intoxications (by history) and the presence of a varicocele (by clinical examination) were ruled out. Their wives underwent a complete fertility investigation revealing no impediment to conception.

All patients were given TL (Teslac®, Squibb B.V., Rijswijk, The Netherlands) 1 gram daily in 2 divided doses and blood for luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactine (PRL), T, E_2 and 17-OHP was collected before and at weekly intervals up to 4 weeks after starting therapy.

Before and at the end of the initial 4 week treatment period, LH-RH (Relefact®, Hoechst Holland, Amsterdam, The Netherlands) 1 µg/minute was administered by constant intravenous infusion (Harvard pump; Harvard Apparatus Company, South Natick, MA) for 180 minutes to all men (10,16).

Blood for LH, FSH, T, E_2 and 17-OHP and PRL measurement was collected at 15-minute intervals by integrated blood sampling through a heparin-coated catheter (Kowarski catheter; Cormed, Middleport, NY) with a constant withdrawal pump (16). The procedure started at t=-30 minutes at 4:00 P.M. Serum LH and FSH were measured by a specific homologous double-antibody solid-phase (DASP) radioimmunoassay using antisera against highly purified hCG and FSH without any significant mutual cross reaction. The intraassay (RIA) variability for duplicate measurements was 8.6% for LH and 8.1% for FSH. Normal ranges in euspermic men are respectively 4 to 15 and 0.9 to 4.5 IU/l (17). Serum E_2 levels were measured by specific RIA with an antiserum raised against E_2-6-(O-carboxymethyl) oxime bovine serum albumin (intraassay coefficient of variation 6.2%) (18). Serum 17-OHP and T concentrations were measured by specific dextran-coated charcoal RIA after extraction with diethylether and subsequent isolation of the 17-OHP and T fractions by Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala Sweden) column chromatography, using antisera raised in rabbits and directed against

17-OHP - 7-carboxyethylthioether - BSA (Radioimmunoassay Systems Laboratories, Carson, CA) and against T - 3-(O-carboxymethyl) oxime - BSA, respectively. Tritiated (1,2,6,7-^{3}H) 17-OHP (Amersham Internationaal; Amersham, U.K.) and (1,2,6,7,16,17-^{3}H) T (New England Nuclear, Boston, MA) were used on the radioactive labels and 17-OHP (range, from 0.025 to 3.0 pmol/tube) and T (sigma Chemical Company, St Louis, MO) (range, 0.01 to 15 pmol/tube) as the standard preparations. Cross-reactivities of a variety of C_{18}, C_{19} and C_{21} steroids were always less than 0.01% (17-OHP), while progesterone and 17α-hydroxypregnenolone showed 0.4 and 1.1% cross-reactivity. In the case of T, cross-reactivities of progestagens and estrogens were always less than 0.05%, whereas 5α- and 5β-dihydrotestosterone showed values of 48% and 14% respectively. Method blanks were below the minimum detectable doses, 0.02 pmol (17-OHP) and 0.007 pmol T per tube at B/B_0 = 0.9. The intraassay and interassay variabilities for means of duplicate measurements were 7.5% and 8.5% for 17-OHP, and 5.5% and 6.5% for T, respectively. Serum concentrations of PRL were measured by specific, homologous, DASP radioimmunoassay. The intraassay coefficient of variation was 6.3%. To avoid interassay variations all samples from one individual were measured in the same assay. Statistical analysis was performed using Student's paired (denoted by P) and unpaired (P*) t-tests, and Freedman's nonparametric analysis of variance (P**).

Unless otherwise stated, the mean values ± 1 standard error of the mean are given.

RESULTS

Effect of TL therapy on basal pituitary-gonadal function

The mean basal LH (10.0 ± 0.7 IU/l) level in the oligospermic men was significantly higher than in an earlier published group of nine healthy controls (7.0 ± 0.8 IU/l, $P^* < 0.02$), whereas the mean serum FSH level tended to be higher (2.5 ± 0.4 IU/l versus 2.0 ± 0.2 IU/l, $0.05 < P^* < 0.10$) (10). In all patients however, serum LH and FSH levels were well within the normal range.

TL therapy within 1 week lowered the mean serum E_2 level ($P < 0.001$ versus baseline) to a minimal value of -33 ± 6% below baseline. Whereas

the mean serum LH level remained virtually unchanged during therapy, the mean FSH level increased (P** < 0.005) within 1 week and thereafter plateaued (fig. 1).

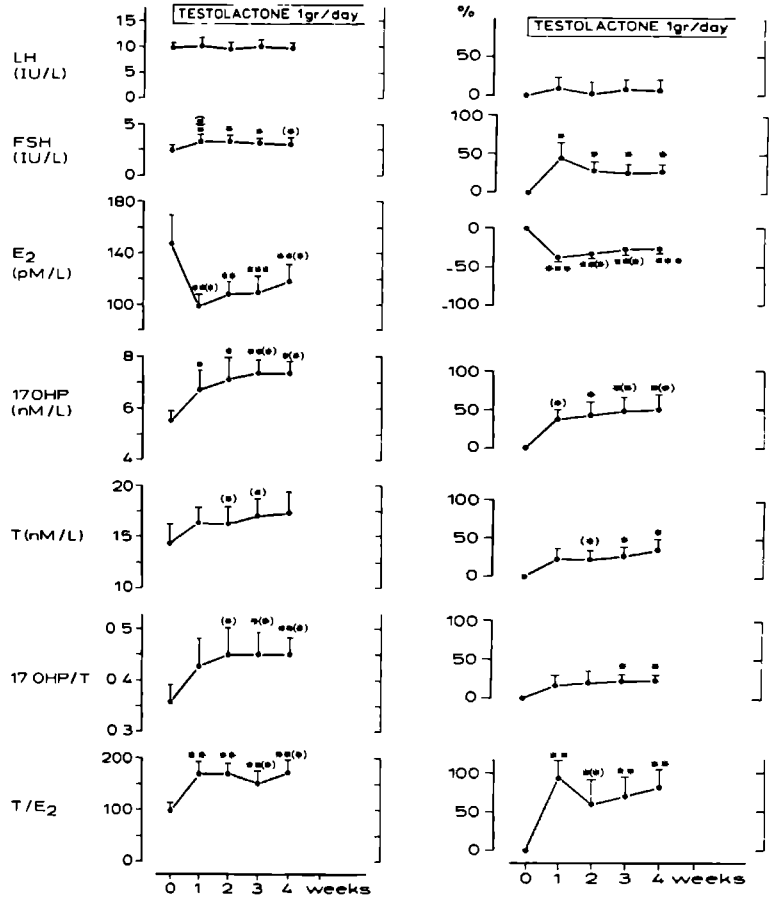

Fig. 1: Effect of 4 weeks TL administration (2 x 500 mg daily) on the mean serum and on the percentage changes vs week 0 of LH, FSH, estradiol (E_2), 17-hydroxyprogesterone (17-OHP) and testosterone (T) levels and the ratios 17-OHP/T and T/E_2 in 9 patients with idiopathic oligospermia. The vertical bars represent SEM, the asterisks the statistical significances: (*) P < 0.10 vs week 0, * < 0.05. *(*) < 0.025. ** < 0.01. **(*) < 0.005. *** < 0.001.

Although there was a tendency for the absolute testosterone (T) levels to rise during TL treatment ($0.05 < P^{**} < 0.10$), at no time interval did the increase reach statistical significance. At 3 and 4 weeks the percentage increases (25 $\pm$ 10% and 34 $\pm$ 14%) were, however, statistically significant. The T/E_2 ratio almost doubled after 1 week of therapy ($P < 0.01$ versus baseline) and remained elevated thereafter.

Serum 17-OHP rapidly increased within 1 week ($P < 0.05$ versus baseline) to a maximum value (1.5 $\pm$ 0.2 times baseline, ($P < 0.025$) after 4 weeks. Because the 17-OHP rise was more pronounced than that of T, the 17-OHP/T ratio significantly increased during TL therapy.

Serum PRL levels did not change throughout the treatment period (data not shown).

Effect of TL medication on LH-RH stimulated pituitary-gonadal hormonal function

Before TL therapy LH-RH infusion for 180 minutes elicited a biphasic LH response with an initial peak at 45 to 60 minutes, a nadir thereafter and a second rise from 120 minutes on ($P^{**} < 0.05$) (fig. 2). The mean serum FSH levels increased within 30 minutes, the maximum being achieved after 125 minutes. Thereafter, the levels plateaued. Serum 17-OHP levels showed an initial fall at 15 and 30 minutes ($P < 0.005$ versus t=0) after starting the infusion, but thereafter the levels significantly increased to a mean value of 1.5 $\pm$ 0.2 times baseline at 180 minutes ($P < 0.02$ versus t=0). The mean serum T levels also showed an initial fall at t=30 minutes ($P < 0.005$), but from 75 minutes on T increased to a maximum value at t=180 minutes. The 17-OHP/T ratio virtually remained unchanged until 45 minutes. Thereafter it showed a tendency to increase until 90 minutes ($0.05 < P < 0.10$ versus t=0) whereafter the levels plateaued. Serum E_2 levels essentially remained unchanged throughout the infusion period.

LH-RH infusion after 4 weeks of TL therapy elicited an LH and FSH response similar to that before treatment, the areas under the curves (LH 223 $\pm$ 47 versus 214 $\pm$ 49, FSH 18 $\pm$ 5 versus 21 $\pm$ 4 area units) being almost identical. As before treatment, there was a tendency to a biphasic LH response.

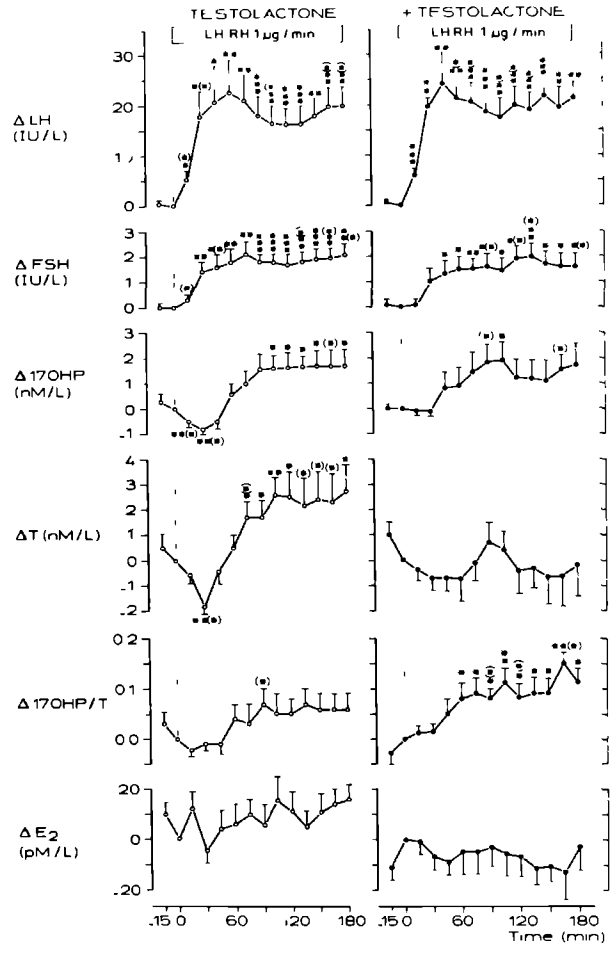

Fig. 2: Effect of 4 week TL administration (2 x 500 mg daily) on the mean LHRH (1 µg/minute for 180 minutes) induced changes versus T=0 in serum LH, FSH, estradiol (E_2), 17-hydroxyprogesterone (17-OHP) and testosterone (T) levels and the 17-OHP/T ratio in 9 patients with idiopathic oligospermia. The vertical bars represent SEM, the asterisks the statistical significances: (*) P < 0.10 vs t=0 min, * < 0.05. *(*) < 0.025. ** < 0.01. **(*) < 0.005. *** < 0.001.

On therapy, serum 17-OHP levels did not show the initial decrease at 15 and 30 minutes after starting the LH-RH infusion observed before treatment. Thereafter, a statistically significant increase occurred.

At no time point before or during therapy did the 17-OHP curves differ significantly from each other, and the areas under the curves (14.9 $\pm$ 6.7 versus 14.8 $\pm$ 4.2 area units) were also similar. In contrast to 17-OHP, serum T levels did not show any significant change throughout the whole LH-RH infusion period. Comparing both T curves before and during TL therapy, at almost all time intervals from 75 minutes on, the T increments in response to LHRH during TL therapy were significantly lower than before TL ($P < 0.05 - < 0.025$), whereas the areas under the T curves (9.2 $\pm$ 4.9 versus 21.4 $\pm$ 5.6 area units, $P < 0.02$) also differed significantly.

During TL treatment, the 17-OHP/T ratio readily increased in response to LH-RH and values significantly higher than baseline were achieved at all time intervals from 60 minutes on. The maximum 17-OHP/T increase in response to LH-RH during TL therapy was significantly higher (0.18 $\pm$ 0.02 versus 0.13 $\pm$ 0.02, $P < 0.05$) than without medication.

The serum E_2 levels on therapy virtually remained the same throughout the LH-RH infusion period as before TL administration.

DISCUSSION

TL medication for 4 weeks lowered the mean serum E_2 levels in patients with idiopathic oligospermia and increased the mean serum FSH significantly, but left serum LH levels virtually unchanged, confirming the data of Leinonen et al. (19), who gave the same dose for only 1 week to patients with prostatic carcinoma. Gooren et al. (5), who administered 1.5 gram of TL daily for 2 weeks reported an LH and FSH response in healthy volunteers similar to that in the present study, whereas Vigersky and Glass (3), using 1 gram daily for 6 to 12 months in patients with idiopathic oligospermia, did not find any change at all, either in FSH or in LH. Other investigators, however, administering higher doses (2 grams daily) for 7 to 10 days to normal male subjects reported a statistically significant increase of both gonadotropins (2,4). The increase in serum FSH levels, but not in LH, in response to the lower doses of TL is probably due to the preferential suppressive effect of endogenous E_2 on pituitary FSH secretion rather than LH (20-22).

Despite the fact that serum LH levels remained unchanged the major

precursor of T, 17-OHP readily increased within one week after starting TL therapy at the same time when serum E_2 levels became significantly decreased. Peak levels of 50% above baseline were achieved after 4 weeks.

In contrast to 17-OHP, serum T levels showed a rather sluggish rise. Literature data on the effect of TL on serum T are conflicting. Marynick et al. (2) reported a clear increase already within 10 days in healthy men treated with a higher dose of 2 gram, whereas others did not find any increase at all (4,5,19). Vigersky and Glass (3), however, administering TL for six to twelve months to oligospermic men, reported a 50% increase of T at the end of the treatment period. Nevertheless most investigators reported an increase of the T/E_2 ratio (2-5).

In the present study the rise in 17-OHP exceeded the serum T increase and therefore the 17-OHP/T ratio significantly increased during treatment with the aromatase inhibitor. This accumulation of 17-OHP over T, which may interpreted to mean inhibition of the 17,20-lyase enzyme system was rather unexpected in the light of the suppressive effect of exogenous and probably also endogenous estrogens on this conversion (11,13,15) and the recent demonstration of alleviation of the block after administration of the competitive estrogen receptor antagonist tamoxifen (14).

A similar relief also was anticipated when serum estrogen levels were lowered by TL, but such relief did not occur. It is hardly intelligible how aromatase inhibition on the one hand enhances 17-OHP and to a lesser degree T secretion by the Leydig cell and on the other hand blocks the conversion of 17-OHP to T. An overall stimulating effect on testicular steroid biosynthesis by the decrease of intratesticular estrogens could be expected, even in the absence of any changes in serum LH levels. Furthermore, it can be argued that the increase of FSH by modulating LH receptor availability may stimulate Leydig cell steroidogenesis. In both concepts however there is no place for an enzyme block.

Another rather unexpected finding was the difference in steroid responsiveness to constant LH-RH infusion before and during aromatase blockade in the present study. The lack of any difference in gonadotropin responsiveness in this and two other studies (3,4) was rather remarkable in the light of the negative feedback effects of estrogens

at the hypothalamo-pituitary level (4,20-22). Nevertheless, despite similar increments in serum LH and FSH, the T response to constant LH-RH infusions with an initial fall and subsequent rise, typical for oligospermia (10) was deleted during TL therapy. In contrast, the 17-OHP response to LH-RH infusion was similar before and during aromatase inhibition. So, again, there was an accumulation of 17-OHP over T, which was even more pronounced than before TL treatment. The data illustrate that in contrast to expectation, TL, despite lowering E_2 levels, did not correct the anomalous Leydig cell response to LH-RH characteristic of oligospermia but rather worsened it with a complete deletion of the T response and aggravation of the 17,20-lyase block. Although these data suggest that E_2 does not play a pivotal role in the genesis of the Leydig cell defect in oligospermia, a direct inhibitory effect of TL per se on Leydig cell steroidogenesis cannot be excluded. Earlier studies suggested that TL had no intrinsic estrogenic or androgenic activity (23). Vigersky et al. (24) recently demonstrated the steroid, in addition to its aromatase inhibiting activity, had antiandrogenic properties (inhibition of the T-induced increase in prostatic weight) by virtue of its competitive interactions with dihydrotestosterone for the androgen receptor (not with the estrogen receptor). This effect is time- and dose-dependent, but doses up to 10 gram daily administered for one month would be needed to exhibit such antiandrogenic effect in men. Furthermore, it is not easily understood how competitive androgen receptor binding of TL would interfere with the conversion of 17-OHP to T. Another explanation is therefore necessary. The aromatase inhibiting effect of TL has been attributed to its competitive binding with T and androstenedione for microsomal cytochrome P 450 (1). Because 17,20-lyase (and 17α-hydroxylase) are also microsomal cytochrome P 450 dependent enzymes (25), it might be speculated that TL would interact as a pseudosubstrate with the cytochrome P 450 leading to enzyme inhibition and accumulation of 17-OHP over T (and also androstenedione (3,19)).

It must be stressed, however, that despite this inhibitory action late in testicular steroidogenesis, which obscures the effect of E_2 lowering, TL overall enhances steroid biosynthesis and leads to almost doubling of the T/E_2 ratio. This latter effect may be beneficial in the treatment of patients with idiopathic oligospermia in whom an altered

testicular estrogen handling has been postulated.

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EFFECT OF CHRONIC AROMATASE INHIBITION BY Δ^1-TESTOLACTONE ON PITUITARY-GONADAL FUNCTION IN OLIGOSPERMIC MEN

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(submitted for publication)

ABSTRACT

Aromatase inhibition by Δ^1-testolactone (Teslac®, 500 mg twice daily) for 6 months in 9 patients with idiopathic oligospermia lowered the levels of serum estradiol (E_2) and thereby sex hormone binding globulin (SHBG) (r_s= +0.40, $P < 0.025$) to values -35 and -25%, respectively, below the pretreatment values ($P < 0.001$ and < 0.005).

The E_2 decrease was accompanied by a temporary increase (+50%) in the levels of follicle stimulating hormone (FSH), not of luteinizing hormone (LH), and of 17α-hydroxyprogesterone (17-OHP), but less of testosterone (T) (+30%), which led to a transient rise in the 17-OHP/T ratio. The T/E_2 ratio and "free T" index (T/SHBG) almost doubled until the end of the treatment period.

During Δ^1-testolactone treatment the mean sperm density gradually rose from 8.1 $\pm$ 1.3 (SEM) before to 21.3 $\pm$ 6.7 x 10^6/ml after 6 months ($P < 0.01$), whereas the total sperm count almost threefold increased ($P < 0.05$). Sperm concentrations exceeding 20 x 10^6/ml were achieved in 4 of the 9 patients. Two of these patients' wives became pregnant.

Although the data point to a pivotal role of estrogens in the pathogenesis of the spermatogenic lesion in some patients with idiopathic oligospermia, the lack of a benificial effect of estrogen lowering in others points to a multicausal nature of the disease entity.

INTRODUCTION

Increased intratubular estrogens or an inordinate sensitivity of the germinal epithelium to their inhibiting effect have been postulated as the cause of the defective spermatogenesis in patients with idiopathic oligospermia (1,2). This inhibitory effect of estrogens on the germinal epithelium may be direct (3) or indirect by inducing an enzymatic block at the 17α-hydroxylase and/or 17,20-lyase locus, thereby interfering with normal Leydig cell steroidogenesis (4-9). Preliminary evidence for a pivotal role of estrogens in the suppression of spermatogenesis was adduced by Vigersky and Glass (1), demonstrating that lowering estradiol levels by administration of the aromatase inhibitor Δ^1-testolactone (TL) (17-oxa-o-homo-1,4-androstadiene-3,17-dione) indeed improves sperm density and fertility in patients with idiopathic oligospermia, without affecting serum gonadotropin levels. In another preliminary study, however, Clark and Sherins (10) failed to demonstrate any effect at all of TL treatment in their patients with idiopathic oligospermia. These conflicting data prompted us to carefully monitor the effect of sustained lowering of endogenous estrogens by TL administration on pituitary-Leydig cell function and sperm parameters in patients with idiopathic oligospermia.

MATERIALS AND METHODS

Nine normogonadotropic oligospermic men (age 30.6 ± 3.9 years standard deviation (SD), range 23-37 year) with longstanding infertility of at least 1 year duaration (2,0 ± 0.7 (SD) years) were studied after obtaining approval of the protocol by the hospital ethical committee and informed consent.

The sperm density in semen samples freshly obtained by masturbation after sexual abstinence for 4 to 5 day was less than 20 x 10^6/ml on at least 3 occasions. The mean sperm concentration was 8.1 ± 1.3 x 10^6/ml range 2.6 to 17.0 x 10^6/ml), the percentage abnormal forms 66 ± 3% and the mean sperm motility 3.3 ± 0.5 (SD) (graded 5 for rapidly progressive and 1 for sluggishly progressive spermatozoa). Testicular volume 19 ± 2 ml (SD) was normal in all patients. Intoxications and testicular inflammatory diseases (by history) and the presence of varicocele were

ruled out in all of them. Their wives underwent a complete fertility investigation revealing no impediment for conception.

All patients were given Δ^1-testolactone (Teslac®, Squibb BV, Rijswijk, The Netherlands) 1 gram daily in 2 divided doses for at least 6 months. Blood samples for luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL), testosterone (T), estradiol (E_2), 17α-hydroxyprogesterone (17-OHP), sex hormone binding globulin (SHBG) and cortisol measurement were collected between 3.00 - 5.00 P.M. before and at monthly intervals up to 6 months after starting therapy. In an earlier study blood samples were also collected at weekly intervals up to 4 weeks and LH-RH responsiveness was assessed before and after 4 weeks of Δ^1-testolactone (TL) treatment. The results of this study will be published elsewhere (11). At monthly intervals after starting TL, semen analyses were obtained as mentioned above.

Serum LH and FSH levels were measured by specific homologous double-antibody solid-phase (DASP) radioimmunoassays using antisera against respectively highly purified hCG and FSH without any significant mutual cross reaction. The intraassay variability for duplicate measurements was 8.6% for LH and 8.1% for FSH. Normal ranges in euspermic men are 4-15 and 0.9 - 4.5 IU/l respectively (11). Serum E_2 levels were measured by specific radioimmunoassay (11) using an antiserum raised against E_2-6 (O-carboxylmethyl) oxime bovine serum albumin (intraassay coefficient of variation, 6.2%) (12). Serum 17-OHP and T concentrations were measured by specific DCC radioimmunoassay after extraction with diethylether and subsequent isolation of 17-OHP and T fractions by Sephadex LH-20 column chromatography, using antisera raised in rabbits and directed against 17-OHP-7-carboxyethylthioether-BSA and against T-3-(O-carboxymethyl)oxime-BSA respectively (12). The intra- and inter-assay variabilities for means of duplicate measurements were 7.5% and 8.5% for 17-OHP and 5.5 and 6.5% for T, respectively.

Serum concentrations of PRL were measured by specific homologous, DASP radioimmunoassay (13) (intraassay coefficient of variation 6.3%).

Serum sex hormone binding globulin (SHBG) levels (nmol/l) were determined using the SHBG immunoradiometric assay (IRMA) test kit manufactured by Farmos Diagnostica (Oulunsalo, Finland). This liquid-phase one-step procedure sandwiches the SHBG analyte between a polyclonal anti-SHBG-antibody raised in rabbits, and a monoclonal SHBG antibody

labeled with ^{125}I. A solid-phase anti-rabbit IgG antiserum is added to precipitate by centrifugation the radiolabeled solid-phase matrix containing the SHBG analyte. The amount of radioactivity in the sedimented solid-phase pellet is directly proportional to the SHBG content in the samples which are interpolated from a standard curve with doses ranging between 6.25 and 200 nmol/l. The sensitivity of the method is 6.25 nmol/l, while its specificity is high. Since no human serum protein is known to cross-react with the sandwich-type antibody combination employed in this assay. The precision of the procedure as tested with three different serum pools (mean values: 11.4, 78.4, and 110 nmol/l) in eight consecutive tests showed an intraassay variability of 4.7% and an interassay precision for means of duplicate measurements between 4.4% and 5.7%. Serum cortisol levels were measured by the ^{125}I-cortisol radioimmunoassay-kit purchased from clinical Assays/Travenol-Grenentech Diagnostics (Cambridge, MA, USA).

To avoid interassay variations all samples from one individual were measured in the same assay. Statistical analysis of the hormonal data was performed using Student's paired (denoted by P) and unpaired (P*) t-tests. The relation between serum E_2 en SHBG levels at the various time intervals was statistically analysed by testing the means of Spearman's rank correlation coefficients (P**). As the sperm counts did not show a normal distribution the Wilcoxon signed rank test was used for statistical analysis (P***).

Unless otherwise stated the mean values $\pm$ 1 standard error of the mean (SEM) are given.

RESULTS

Effect of chronic testolactone treatment on the pituitary Leydig cell function (figs. 1-3)

The mean basal LH level in the oligospermic men (10.0 $\pm$ 0,7 IU/l) was significantly higher than the mean concentration in an earlier published (14) group of healthy controls (7.0 $\pm$ 0.8 IU/l, $P^* < 0.02$), whereas the mean FSH level tended to be higher (2.6 $\pm$ 0.4 vs 2.0 $\pm$ 02 IU/l, $0.05 < P^* < 0.10$. In all patients however, serum LH (7.2 - 13 IU/l) and FSH (0.9 - 4.2 IU/l) levels were within the normal range. The

mean basal serum T (14.6 ± 1.8 versus 11.1 ± 1.1 nmol/l and E_2 (159 ± 20 versuss 129 ± 10 pmol/l) did not differ significantly between the two groups.

TL treatment lowered serum E_2 levels significantly ($P < 0.001$) within 1 month to lowest values 34 ± 10% below baseline at 3 months (fig. 1).

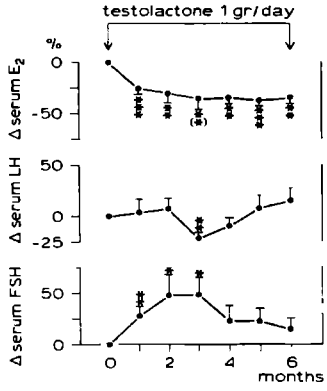

Figure 1: Effect of Δ^1-Testolactone administration (Teslac®, Squibb, Rijswijk, The Netherlands, 500 mg twice daily) for 6 months on the mean percentage changes of serum E_2, LH and FSH levels in 9 patients with idiopathic oligospermia. The asterisks indicate the statistical significance of the changes as compared to the baseline value. * $P < 0.05$. *(*) $P < 0.025$. ** $P < 0.01$. *** $P < 0.001$.

Afterwards the levels remained at this level (-33 to -35%) during the next 3 months ($P < 0.01$ - < 0.001 versus baseline). The mean serum LH levels did not change during the 6 months treatment period, except for an unexplained slight but significant decrease at 3 months ($P < 0.02$ versus t=0). The mean serum FSH levels increased significantly from 1 month on ($P < 0.01$ versus t=0) to peak values 1.5 ± 0.2 times the baseline after 2 and 3 months ($P < 0.05$). Afterwards the mean FSH levels fell to values not significantly different from baseline.

The mean total serum T levels significantly increased to peak values 1.34 ± 0.1 times the baseline ($P < 0.05$ versus t=0) 1 month after starting therapy and thereafter gradually declined to levels (1.1 - 1.2 times the baseline) not significantly different from the pretreatment value.

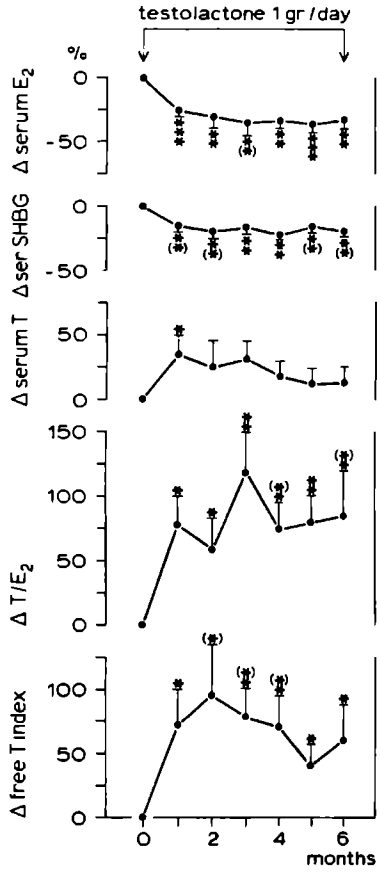

Figure 2: Effect of Δ^1-testolactone administration (2 x 500 mg daily) for 6 months on the mean percentage changes of serum E_2, sex hormone binding globulin (SHBG), T, the T/E_2 ratio and the "free" T index in 9 oligospermic men. The asterisks indicate the statistical significances of the changes as compared to the baseline value. (*) $0.05 < P < 0.10$. * $P < 0.05$. *(*) $P < 0.25$. ** $P < 0.01$. *** $P < 0.001$.

When the T values however were corrected for the significantly decreasing SHBG levels (nadir value -22 $\pm$ 4% below baseline at 4 months, $P < 0.005$) the "free" T index (T/SHBG) rose to values 1.4 to 2.0 times the baseline ($P < 0.025$ - < 0.005) throughout the treatment period.

The T/E_2 ratio also significantly increased within one month ($P < 0.02$) and remained significantly elevated at a level 1.6 to 2.2 times

the pretreatment value ($P < 0.05 - < 0.005$) until the end of the treatment period. Except for a statistically significant fall of one month ($P < 0.01$ versus baseline) the E_2/SHBG ratio remained unchanged throughout the treatment period. Serum E_2 and SHBG levels were significantly correlated with each other ($r_s = 0.040$, $P^{**} < 0.005$).

The mean serum 17-OHP level significantly increased within one month to a value 1.5 $\pm$ 0.2 times the baseline ($P < 0.025$), thereafter plateaued until 3 months and then gradually fell to values not significantly different from baseline (fig. 3).

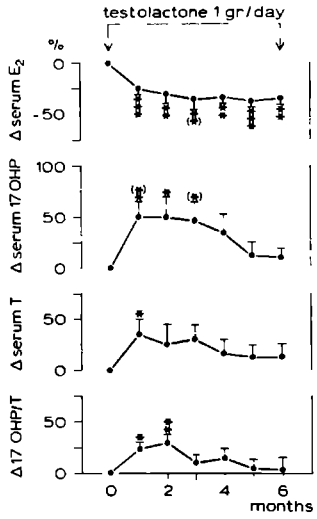

Figure 3: Effect of Δ^1-testolactone administration (2 x 500 mg daily) for 6 months on the mean percentage changes in serum E_2, 17-OHP, T and the 17-OHP/T ratio in 9 oligospermic men. The asterisks indicate the statistical significance of the changes as compared to the baseline value. (*) $0.05 < P < 0.10$. * $P < 0.05$. *(*) $P < 0.025$. ** $P < 0.01$. *** $P < 0.001$

As the 17-OHP increase exceeded the rise of T, the 17-OHP ratio to T transiently significantly increased to 1.2 and 1.3 times the pretreatment value 1 and 2 months after starting therapy ($P < 0.05$ and < 0.005 respectively). Thereafter the 17-OHP/T ratio gradually declined to baseline values.

The mean serum prolactin and cortisol levels did not change significantly throughout the treatment period (data not shown). There were no systematic changes in hematological, liver or renal function parameters during TL therapy nor did the patients mention any complaints.

Effect of chronic Δ^1-testolactone treatment on sperm parameters (fig.4)

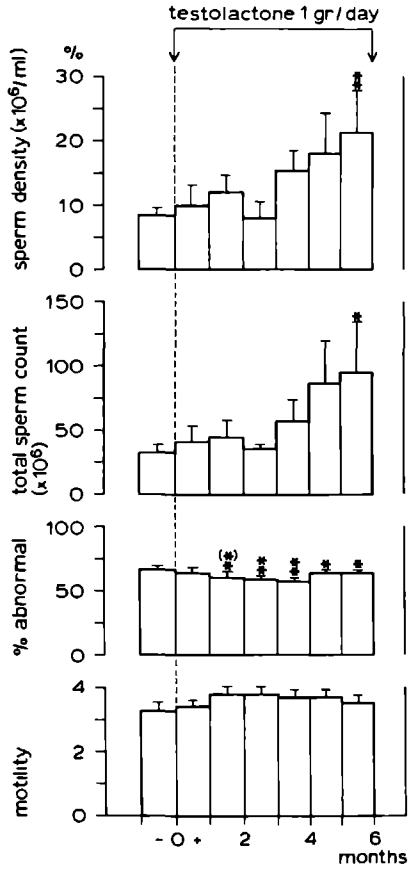

Figure 4: Effect of Δ^1-Testolactone administration (2 x 500 mg daily) for 6 months on the mean sperm density, total sperm count, percentage abnormal forms and motility in 9 patients with idiopathic oligospermia. The asterisks indicate the statistical significance of the changes as compared to the pre-treatment values. * $P < 0.05$. *(*) $P < 0.025$. ** $P < 0.01$

The mean sperm density did not increase significantly during the first 3 months of TL treatment (8.1 ± 0.8 to $11.9 \pm 2.8 \times 10^6/ml$).

Thereafter the mean concentration gradually increased but a statistically significant rise did not occur until 6 months after starting therapy (21.3 $\pm$ 6.7 x 10^6/ml, P*** < 0.01 versus pretreatment value). In 5 of the patients sperm density increased by more than 2 SD above the baseline values, whereas sperm counts exceeding 20 x 10^6/ml were achieved in 4 of the patients. Two of these patients' wives conceived.

The total sperm count also remained unchanged during the first 3 months of treatment. Thereafter, the mean values gradually rose to significantly higher total sperm counts (95.9 $\pm$ 38.6 x 10^6 versus 32.8 $\pm$ 5.3 x 10^6/ml, P < 0.05) at six months. In 5 patients total sperm counts increased by more than 2 SD above the baseline value. The percentage abnormal forms slightly but significantly decreased in 7 of 9 patients from 2 months on until the end of the treatment period (P < 0.025 - < 0.005 versus t=0). There was no significant change in sperm motility.

DISCUSSION

The administration of TL for six months to 9 oligospermic men produced a sustained decrease of serum E_2 and thereby SHBG concentrations to values about 35 and 25% respectively below the pretreatment level, increased the free T index and T/E_2 ratio by about 100% and almost doubled the mean sperm density. The decrease in serum E_2 levels induced by the aromatase inhibitory effect of TL was accompanied by a transient increase in serum FSH levels - not however LH - which reached a maximum after 2 and 3 months and then faded out despite persistence of the E_2 lowering. Such temporary rise in FSH was not mentioned in the long term TL study of Vigersky and Glass (1), who in contrast stressed the lack of any change in FSH as well as LH in their patients treated for 6 to 12 months. The increase in FSH levels, but not LH, in the present study may be due to preferential suppression of the tonic inhibitory effect of endogenous estrogens on pituitary FSH secretion by the aromatase inhibitor (14).

The fall of FSH after the initial increase is, however, more difficult to explain. Other authors (16,17), treating patients with idiopathic oligospermia with clomiphene or tamoxifene, observed a similar decline in serum FSH 3 months after starting therapy, which they

ascribed to the concomitant activation of the spermatogenic activity and probable concomitant feedback inhibition by inhibin. In the present study sperm density admittedly increased from 3 months on, but a statistically significant increase was not obseved until 6 months.

The lowering of serum E_2 levels was accompanied by a significant rise of total T and its major precursor 17-OHP. It is not quite intelligible how TL increases testicular steroid biosynthesis in the absence of an enhancing effect on serum LH levels. An overall stimulating effect on Leydig cell steroidogenesis might, however, be explained by a decrease in intratesticular estrogens which have a tonic suppressive effect on the steroidogenic key enzymes, 17α-hydroxylase and 17,20-lyase and thereby interfere with T synthesis (4-9). Remarkably however is the fact that the increase in serum 17-OHP exceeded the rise in T and therefore a transient increase in the 17-OHP over T ratio was observed 1 and 2 months after starting TL therapy. This accumulation of 17-OHP over T which may reflect a temporary blockage at the 17,20-lyase locus we earlier tentatively ascribed to be caused by an intrinsic inhibitory effect of TL itself on this enzyme (11). The relieve of this enzymatic block thereafter may be due to the preponderance of the facilitating effect of E_2 lowering per se over the inhibitory effect of TL.

It has to be noted that both the 17-OHP and T increments were apparently evanescent, despite persistence of the E_2 lowering. As far as T is concerned the subsequent decline can readily be explained by the not earlier reported concomitant fall in SHBG levels which closely correlated with E_2. After correction for this decrease the "free" T index remained significantly elevated which was further corroborated by the rise in salivary testosterone (to be published). The decline in 17-OHP levels may also be due to an estrogen mediated decrease in its major binding globulin, cortisol binding globulin (CBG), but this seems unlikely as serum cortisol levels did not significantly fall during TL therapy. As suggested earlier the decrease in 17-OHP can also be explained by the enhancing effect of sustained estrogen lowering on the enzymes involved in testosterone biosynthesis.

Nevertheless aromatase inhibition by TL and E_2 lowering led to an increase in the T/E_2 ratio and "free" T index which - together with the temporary rise in serum FSH - may have been responsible for the improvement in spermatogenesis and possible fertility in the present

study.

Estrogens inhibit directly spermatogenesis in rats (3) and probably also in men (2,18). Elevated spermatic vein and peripheral E_2 levels have been found in patients with germinal cell arrest (2), and in infertile men with elevated levels of FSH (18). Furthermore, Sherins and Clark (19) demonstrated that excessive E_2 levels prevented completion of spermatogenesis in hypogonadotropic patients treated with human chorionic gonadotropin alone and that normalization of estradiol by the aromatase inhibitor TL restored spermatogenesis. As intratesticular T levels have been reported to be lowered in idiopathic oligospermia (20) the increase in T and the T/E_2 ratio also may have contributed to the improvement of spermatogenesis in the present and Vigersky and Glass' study (1). In both studies mean sperm density almost doubled and pregnancy occurred in 3 respectively 2 of the patients' wives.

A slight but significant decrease in the percentage abnormal forms was found in the present study whereas no changes were seen in motility.

In contrast to both these encouraging reports, a very recent preliminary study of Clark and Sherins (10) failed to demonstrate any effect at all of TL on total sperm counts, motility, or morphology in a placebo controlled randomized double-blind cross-over study. During 8 months of therapy the mean total sperm counts remained unchanged in both groups of patients treated with either placebo or 2 gram of TL daily. Unfortunately no hormonal data are given on the effect of both drug regimens throughout the treatment period.

Comparing the effect of the competitive E_2 receptor antagonist tamoxifen with that of the aromatase inhibitor TL on sperm density in patients with idiopathic oligospermia the differences in hormonal effects have to be taken into account. In contrast to TL tamoxifen produces a rise in both serum LH and FSH levels and equally increases T and E_2, leaving the T/E_2 ratio unchanged (17,21-23). Nevertheless both agents are equally effective in improving spermatogenesis and fertility in oligospermic patients, as doubling of the sperm density was also achieved in most reports dealing with the effect of tamoxifen (17, 21-23). In an attempt to combine the beneficial effects of both drugs, Vigersky and Glass (24) failed to notice any further improvement of sperm density above that achieved with TL alone.

The results obtained with E_2 lowering through aromatase inhibition are encouraging and are in favour of a pivotal role of estrogens in the genesis of the spermatogenic lesion in idiopathic oligospermia. However, the lack of such beneficial effect in still a substantial percentage of these patients (about 40% in ours and Vigersky and Glass' study) points to a multicausal origin of the lesion and pleads for better selection criteria and search for more effective treatment modalities in these patients.

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DISCUSSION AND CONSIDERATIONS

In the preceding chapters attention is paid to diagnostic and therapeutic aspects of idiopathic oligospermia. In chapter VI the data and conclusions of the preceding chapters will be integrated and followed by a general discussion on testicular function. Suggestions which may contribute to further progress in the development of more effective diagnostic and therapeutic modalities in patients with idiopathic oligospermia are also presented.

From the data in chapter II it appears that in men with idiopathic oligospermia there is a state of compensated Leydig cell failure as basal plasma testosterone levels are normal but only at the cost of supranormal serum gonadotropin levels, which confirms data of other authors (1-9). Under basal circumstances no accumulation of the major precursor of testosterone, 17α-hydroxyprogesterone (17α-OHP) was observed in the oligospermic patients, which contrasts with in vivo and in vitro data of Steinberger et al. (10) and Rodriguez-Rigau and coworkers (11). Their data demonstrated evidence of a 17,20-lyase block in oligospermic men. Plymate et al.(12) also reported elevated 17α-OHP and lowered testosterone levels in patients with idiopathic oligospermia as compared to eugonadal men.

In the present study, however, only after LH-RH stimulation indirect evidence of a 17,20-lyase block was obtained which agrees with the data of Ando et al. (13,14) in oligospermic patients with varicocele. Earlier we reported the presence of such a block in gonadotropin stimulated patients with Klinefelter's syndrome (15). In addition to the occurrence of this key enzymatic block late in steroidogenesis, LH-RH infusion revealed the absence of the initial testosterone rise present in eupermic men 15 to 30 minutes after starting the infusion. Furthermore, the late rise in testosterone was only half that in the controls (16).

It has been known for some years that both in rats and man estrogens play a permissive role in the occurrence of the late steroidogenic block (Chapter I). In the oligospermic patients from the present study, serum estradiol (E_2) levels were higher than in the eugonadal men (42

versus 35 pg/ml), but this difference lacked statistical significance. In patients with severe tubular damage an increase in peripheral and spermatic estradiol levels has been reported (17-19). In patients with idiopathic oligospermia raised intratesticular estrogen levels have been postulated as the cause of the gonadal dysfunction (20). We therefore hypothesized that these increased E_2 levels may be the cause of the anomalous Leydig cell response to LH-RH infusion in these patients. Therefore lowering of this endogenous estrogen increase by chronic administration of the aromatase inhibitor Δ^1-testolactone (Teslac®) could correct the anomaly. Our results, however, were rather disappointing in this respect. Indeed Δ^1-testolactone administration for 1 month and longer led to a 30% decrease in circulating serum E_2 levels with enhancement of the FSH, 17-OHP and T secretion. Both under basal and stimulated conditions, however, an aggravation of the 17,20-lyase block occurred, instead of the expected relieve. Furthermore, administration of Δ^1-testolactone completely deleted the late testosterone response to LH-RH infusion, whereas the lack of the initial testosterone response persisted. Together these data therefore suggest that estrogens do not play a significant role in the genesis of the Leydig cell defect in oligospermia. Remarkable however, is the fact that in eugonadal men Δ^1-testolactone administration for 1 week also led to an aggravation of the 17,20-lyase block, both under basal and gonadotropin (hCG) stimulated conditions with a similar blunting of the testosterone response to hCG and accumulation of 17α-OHP (21). In an earlier study, administration of the competitive estrogen receptor antagonist tamoxifen appeared to completely abolish the hCG induced 17,20-lyase block in normal men (22). The data on ^{1}-testolactone in the oligospermic men therefore do not favour, nor exclude a permissive role of estrogens in the genesis of the Leydig cell abnormality in severe tubular damage. They only point out to an hitherto unknown intrinsic action of Δ^1-testolactone per se - apart from its aromatase inhibiting action - on probably the microsomal enzymes late in steroidogenesis (chapter IV). This intrinsic effect may have obscured the expected beneficial effect of reducing E_2.

Unfortunately, in the tamoxifen study in the oligospermic patients no 17α-OHP levels could be measured. Therefore the question of the estrogen dependency of the 17,20-lyase block induced by LH-RH under

basal conditions remains unanswered. Scrutinizing the data on testosterone one month after starting the E_2 receptor antagonist (chapter IV) the early T increase in response to LH-RH infusion was still absent, whereas the late response was similar to that before tamoxifen administration. These data suggest that the early defect is not estrogen dependent or at least is not mediated by the Leydig cell estradiol receptor. We, therefore, have to hypothesize that the lack of an early testosterone response to LH-RH in idiopathic oligospermia is due to the absence of a rapidly releasable pool of precursor steroids or testosterone in the Leydig cells of these patients. It has to be demonstrated whether longstanding hypergonadotropism per se or the induction by local factors produced by the Sertoli cells and interacting with LH to modulate Leydig cell function, is responsible for the lack of the initial testosterone response in idiopathic oligospermia (23-33).

In the introduction to this thesis we alluded to the possible dose dependency of tamoxifen therapy on the final outcome of sperm parameters in idiopathic oligospermia.
From the data in chapter III it is clear that lower and higher doses of tamoxifen are equally (although only modestly) effective in improving sperm density and total sperm output without affecting motility or morphology. This concordance in stimulating effect of both lower and higher doses of tamoxifen could already be presaged from the similarity in basal and LH-RH stimulated gonadotropin and steroid responses present one month after starting tamoxifen. These data therefore suggest that if tamoxifen per se has any effect on spermatogenesis - Frajese (34) indeed found good evidence for such effect in a placebo controlled double blind cross-over study - this effect is not dose dependent or it has to be assumed that the lower dose of 5 mg or 10 mg is already at the plateau of the dose response curve of this drug. If this is true, we cannot explain the failure of Willis et al. (35) to observe any beneficial effect of 10 mg tamoxifen on sperm parameters in their patients with idiopathic oligospermia or it must be due to selection of patients with a more severe degree of oligospermia.

Scrutinizing the hormonal changes induced by tamoxifen in the oligospermic patients from the present study no changes in the testosterone over estradiol ratio were observed as both steroids showed a similar increase. In view of the hypothesized increased intratubular estrogen

concentrations and/or inordinate sensitivity of the germinal epithelium to their inhibiting effect, theoretically an increase in testosterone and lowering of estradiol would be a more desirable effect when treating patients with oligospermia. Six months of estradiol lowering by administration of the aromatase inhibitor Δ^1-testolactone (chapter V) significantly decreased serum sex hormone binding globulin levels and almost doubled the free testosterone index and T/E_2 ratio. This was accompanied by a temporary rise in FSH and an increase in both sperm density and sperm counts. This improvement started after four months of treatment at the time when FSH levels fell, but only became statistically significant after six months. In the first three months no effect of Δ^1-testolactone treatment on sperm indices was observed. This was to be expected in view of the time needed for sperm maturation and the transit time through the epididymis. The results obtained in the present study are similar of those of Vigersky and Glass (20) but contrast to those of Clark and Sherins (36) who did not observe any change in sperm parameters neither with placebo nor with - the higher dose of 2 gram daily - Δ^1-testolactone.

Although the results obtained with both the E_2 receptor antagonist tamoxifen and the aromatase inhibitor testolactone are encouraging and favour a role of estrogens in the genesis of the spermatogenic lesion in some patients with idiopathic oligospermia, the lack of a beneficial effect in others, points to a multicausal nature of the disease entity. The data therefore plead for better selection of patients with idiopathic oligospermia.

As shown in this study the determination of basal hormone concentrations and stimulated hormone concentrations in peripheral venous blood alone has not proved an appropriate method for optimal selection. Moreover several authors reported an extraordinary <u>high</u> intratesticular T levels in oligospermic patients despite low peripheral T concentrations (37,38,39,40). As 75% of the peripheral estradiol concentration is due to extratesticular conversion of testosterone into estradiol (41) only determination of the spermatic vein or intratesticular concentration of this steroid will give valid information. There is therefore a need to measure intratesticular hormone levels to obtain more insight in human spermatogenic (patho)fysiology. An exhaustive knowledge of the physiological interactions beteen Leydig cells, Sertoli cell and germ cells

is necessary. Up till now this knowledge is almost only available from experience in rodents. Recent studies in the rat suggested that the seminiferous tubules and probably the Sertoli cells secrete factor(s) which locally regulate Leydig cells activity. In 1978 Aoki and Fawcett showed that focal tubular damage, caused by implantation of a silastic tube with anti-androgen in the rat testis, results in hyperplasia of the Leydig cells in the vicinity of the implant (42). Leydig cells located in immediate vicinity of the tubular wall were larger in size than perivascular Leydig cell which are closest to circulatory LH (43). This was especially true for stage VII and VIII of the rat spermatogenic cycle (meiosis), when endogenous testosterone concentration and secretion of androgen binding protein (ABP) are close to maximal value. Therefore it seems that in rats the tubules have a stimulatory influence on the Leydig cell which among others is dependent on the stage of the spermatogenic cycle. The nature of this paracrine influence is unknown. In abdominal testes, tubular damage abolishes this stage dependent variation in the size of the paratubular Leydig cells whereas the sensitivity to human chorionic gonadotropin is reduced (44,45).

There are compelling reasons for believing that Sertoli cell-Leydig cell communications are an integral part of normal testicular function. As yet there is little information on the nature of the factor(s) which facilitate(s) seminiferous tubule-Leydig cell interaction. One candidate is testicular LH-RH or LH-RH-like factors produced by the Sertoli cell and exclusively acting on the Leydig cell (23-30) through its LH-RH (like) receptor. In this respect it is relevant that Leydig cells from testes with damaged tubules show a greatly enhanced response to LH-RH in vitro, as do Leydig cells deprived of gonadotropin stimulation for longer periods. In 1984 Sharpe et al.(30) adduced evidence for the presence of a high molecular weight factor in rat testicular interstitial fluid with major stimulatory effect on Leydig cell steroid secretion. Verhoeven and Cailleau (31) using different approaches, came to the same conclusion: Sertoli cells secrete a factor stimulating an early step in the steroidogenic pathway but inhibiting the conversion of C_{21} into C_{19}-steroids. These effects resemble those observed with exogenous LH-RH, but in contrast to the latter, the activity of the Sertoli cell factor was not inhibited by an LH-RH antagonist.
Parvinen et al. (46) also reported, that the stage dependent Leydig

cell stimulation observed predominantly in stage VII and VIII of the rat seminiferous epithelial cycle, could not be blocked by a LH-RH antagonist. In addition to a stimulatory effect of the Sertoli cells the same investigators adduced evidence for the secretion of a factor partly inhibiting testosterone secretion by Leydig cell (47). In this way Sertoli cells exercise a fine local tuning control on testicular testosterone levels, which is more efficient than the feedback regulation via the hypothalamic-pituitary axis. The intratesticular secretion of factors with major effects on Leydig cell steroidogenesis, has obvious implications in view of the absolute and quantitative dependence of spermatogenesis on the extraordinary high intratesticular testosterone levels (48).

Reflecting the altered Leydig cell function during the spermatogenic cycle in the rat, enzymes present in the peritubular Leydig cells and germ cells also show cyclic changes. Thus in the rat highest levels of acid phophatase activity - generally thought to reflect uptake of testosterone - is found at the stage of mid-pachytene spermatocytes. At this stage testosterone dependency is highest and production of its carrier androgen binding protein (ABP) is maximal (49,50). Similarly levels of the microsomal enzyme 3β-hydroxysteroid-dehydrogenase (3β-HSD) in the peritubular Leydig cells, which converts Δ^5 to Δ^4 steroids, are highest in this stage (49,50).

Although the human male seminiferous tubules have an atypical arrangement of the spermatogenic epithelium, acid phosphatase activity shows a similar behaviour as in the rat. In the human this activity also arises at the mid pachytene spermatocyte stage (50,51). In cases of spermatogenic damage (spermatogenic arrest, Sertoli cell only syndrome), levels of acid phosphatase are decreased concomitantly with diminished levels of testosterone and the enzyme 3β-HSD (51). The infertile men with low 3β-HSD activity in vitro may be beneficially treated with exogenous gonadotropins to stimulate testosterone steroidogenesis, thereby providing the high intratesticular concentration of testosterone essential for normal spermatogenesis (52-54). These data illustrate that also in man there is evidence for a stage dependent cycle of the Leydig cell function and probably also of the Sertoli cell.

In the rat diminished secretory capacity of the Sertoli cell has

been found in all states of spermatogenic damage (cryptorchidism, fetal irradiation, vitamin A deficiency, hydroxyurea treatment) (55,56). Impairment of Sertoli cell function has also been shown in naturally occurring models of diminished spermatogenesis. In the H^{re} rat with oligospermia and elevated FSH levels, production of ABP by the Sertoli cell is diminished (57).

Apart from impaired Sertoli cell function, Leydig cell function is also impaired in animal and human spermatogenic damage, as reflected by lowered circulating testosterone levels, a diminished response to hCG and elevated levels of LH with an exaggerated rise following LH-RH (16,56,58-65). This interference with Leydig cell function occurs in rats, rams, and in men, indicating a common response to spermatogenic damage (55,56). The cause of this change in Leydig cell function associated with spermatogenic injury is not understood. It is necessary to postulate that the seminiferous tubules exert a controlling influence on Leydig cells which is disrupted following spermatogenic damage. The nature of this interaction is unknown, but again substances with LH-RH-like activity have been thought to be involved as intercellular messengers between the tubules and the intertubular tissue (23-31,46). In man also the presence of LH-RH-like activity has been demonstrated in seminal plasma (32,33). It is remarkable however, that in some clinical condition (Klinefelter's syndrome (66), Sertoli cell only syndrome) the spermatogenic damage is associated with hypertrophy of the Leydig cells. In adult rats a similar hypertrophy of Leydig cells has been demonstrated after any type of spermatogenic disruption. Yet despite this hypertrophy, the testosterone response to hCG stimulation is subnormal at least in vivo. In vitro the biosynthetic capacity of the Leydig cell tested by hCG was diminished at lower doses but surprisingly increased at higher doses (56,57).

From the foregoing, it appears that there is a close interaction between Sertoli cells and Leydig cells in rats and probably also in man. Receptors for testosterone have been shown in Sertoli cells (67) which together with FSH has been reported to increase the secretion of ABP amongst others. ABP thereby provides the high intratesticular testosterone microenvironment necessary for spermatogenesis (48).

In addition to the close relationship between Leydig cells and Sertoli cells (67-69), there is also a close interaction between

Sertoli cells and germ cells and visa versa (69,70). First the Sertoli cells provide pyruvate and lactate as energy substrate to the germ cells (71,72). The production of which is markedly increased by FSH, testosterone and also insulin. Furthermore, at least in rats the Sertoli cells have the capacity to metabolize lipids and this may represent the primary source of energy for the Sertoli cell and additional energy substrate to the germ cells (73). Besides ABP and LH-RH-like factors (74,75), a number of other testis specific proteins supporting spermatogenesis is produced by Sertoli cells, providing the optimal microenvironment for the fulfillment of the various stages of spermatogenesis such as plasminogen activator, transferrin, meiosis inducing (MIS) and preventing substances (MPS) which also have been demonstrated in the human male (76,77).

It has been mentioned earlier that spermatogenic cells influence Sertoli cell function, since Sertoli cells adjacent to different stages of spermatogenic epithelium display different properties with respect to production of plasminogen activator, ABP (78), MIS and MPS (77), enzymatic activity (acid phosphatase) (79), and binding of FSH (80).

From all these foregoing arguments it is clear that there is a close interrelationship between Sertoli cells on one hand and Leydig and germ cells on the other. This relationship emphasizes that the testis should be considered as a functional unit and not as 3 independently functioning compartments. Every proces interfering with one of these three compartments therefore must have deleterious implications for the others and lead to disruption of Leydig- and Sertoli-cell function and impairment of spermatogenesis.

Unfortunately hitherto knowledge on the interaction between the three compartments in man is only fragmentary as compared to that in rats. Admittedly there is ample evidence in man for a defect in Leydig cell function in patients with spermatogenic damage (56,58-65), although it is still unknown whether the primary defect resides in the Leydig cell or in the seminiferous tubule.

A simple comparison of testicular function between man and rat is not possible despite the resemblence in many aspects.

Human Leydig cells as compared to rat Leydig cells have a low amount of LH receptors. They contain less than 10% of the same receptor in the rat Leydig cell (81). Gonadotropin stimulation increases human testicu-

lar steroid biosynthesis only marginally (less than 50%), in contrast to the tenfold increase in the rat. This may be due to the insufficient supply or further metabolism of mitochondrial cholesterol in man (81). The most striking difference between rat and human testis is the Leydig cell density. Only 1% of the testicular volume of the rat consists of Leydig cells whereas in man this is as high as 12%. The difference in Leydig cell density is compensated by the large functional activity in the rat. Furthermore differences in steroid synthesis exist between rats and men. In men in contrast to rodents and some subhuman primates (orangutan and rhesus monkey) the ^{5} pathway is preferred for the synthesis of testosterone with a somewhat lower conversion of testosterone occurring through the Δ^4 pathway (82). Whereas the presence of LH-RH receptors on the Leydig cell in rats has been firmly demonstrated, its presence on human Leydig cells is still a matter of controversy (83).

With respect to spermatogenesis overt differences between rats and man are present. In rat the complete cycle lasts 12 days and comprises at least 14 stages, each stage being composed of a specific cellular constellation representing a particular degree of maturation. In man the spermatogenic cycle comprises only 6 stages and lasts 74 $\pm$ 4.5 days (84). In rats and most mammalian species there is a wave like arrangement of the seminiferous epithelium which can be visualized by transillumination of freshly isolated unstamed seminiferous tubules. The human male is a rare exception to this rule, as, there is a highly disorganized pattern of distribution of the various stages of spermatogenesis. The arrangement is patchy and mosaic-like, two or more stages of spermatogenesis occurring together in a single cross-section of the tubule. Nevertheless also in man stage dependent changes in Leydig cell and Sertoli cell enzymatic activities have been observed.

In rats, a species without sex hormone binding globulin (SHBG), the presence of ABP, the intracellular carrier of testosterone and dihydrotestosterone has been convincingly demonstrated. In man the presence of this macromolecule has not been conclusively reported: ABP and SHBG being structurally and immunologically similar (85-88).

From the foregoing it is clear that there are major differences in testicular steroidogenesis and spermatogenesis between man and rat. Simply extra-polating data obtained in rats to the human therefore may be hazardous, despite the fact that Rodriguez-Rigau et al in 1980

stated that "current concepts of hormonal regulation of mammalian spermatogenesis derived primarily in the rat, also apply to the human testis" (89).

However, even if a laboratory animal model exists with a spermatogenesis more comparable to man, still the problem of oligospermia would not have been solved. This is due mainly to two factors: Firstly the definition of oligospermia is too unspecific and allows great variability and secondly, the etiology of idiopathic oligospermia is far from homogenous. The definition of oligospermia in most of the studies is based on sperm density, whereas the total sperm count of an ejaculate is a more reliable parameter. The total sperm count eliminates the pseudo-oligospermia which can be due to a large ejaculate volume. As nearly all sperms are present in the first fraction of an ejaculate collected in two equal volume fractions there is the possibility that incomplete collection of semen can influence the calculated sperm density (90). This has not only serious consequences for a proper diagnosis of oligospermia, but also for a proper interpretation of the results of therapeutical regimens.

A different problem is the question how often a semen analysis must be obtained before a proper diagnosis of oligospermia can be made. Six semen analyses over a period of 3 months lower considerably the risk of a faulty diagnosis of oligospermia due to, for an example, a temporary depression of spermatogenesis caused by a viral infection.

The diversity in the etiology of idiopathic oligospermia constitutes however the most important problem. Kjessler (91) showed for instance, that in men with oligospermia, despite a normal karyotype in cultured lymfocytes from peripheral blood, an aberrant karyotype could be discovered in studies on meiotic division in material obtained from testicular biopsies. Rabin et al. (92) found in a group of 24 patients with oligospermia in 75% of them immune deposits in their testis biopsies. However the significance of these immunologic findings is uncertain. Recently, Yoshida et al. (39) reported on the significance of paying as much attention to the tubualr compartment as to interstitial compartment of testis biopsies underlining the need to see these compartments as a functional unit: They combined and integrated the hormonal, histological and biochemical findings in oligospermic men. This made it possible to divide men with the same degree of oligosper-

mia, tubular derangement and serum FSH levels in two categories. One category was characterized by dense clusters of proliferative Leydig cells, normal serum testosterone levels, but elevated serum LH levels and higher intratesticular testosterone concentrations and steroidogenic enzyme activities. The other category showed sparse Leydig cells in combination with lower serum testosterone and LH levels, in combination with lower intratesticular testosterone concentrations and steroidogenic enzyme activities. It was concluded that only the last category could benefit from Leydig cell stimulating therapy.

From the foregoing, it seems obvious that progress in the knowledge of the pathofysiology of idiopathic oligospermia asks for intensive investigation in the individual patient. The current detailed knowledge on the pathofysiology of female reproductive impairment and the great progress in therapeutic modalities has been achieved due to timely investments in patients and volunteers. As nearly fifty per cent of marital infertility is due - at least in part - to impaired male fertility, there is a strong need to invest more research in this area of impaired male infertility.

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Impairment of male fertility is a common cause of barren marriage. Oligospermia is quite often the reason of subfertility in man. In the majority of the moderately oligospermic patients no definite cause of the oligospermia can be detected and the final diagnosis is "idiopathic oligospermia". The studies presented in this thesis concern patients with idiopathic oligospermia.

In chapter I oligospermia is defined according to MacLeod and Gold: arbitrarily the sperm density of 20 x 10^6/ml was chosen to differentiate between fertile and subfertile men. In all patients, in addition to the oligospermia, there are also decreased sperm motility (asthenozoospermia) and an abnormal morphology (teratozoospermia).

In this chapter the multicausal nature of oligospermia has been stressed and the criteria for the diagnosis idiopathic oligospermia have been assessed. It appears that idiopathic oligospermia is not a distinct nosological entity but rather a mixture of hypothalamic, pituitary and gonadal abnormalities leading to spermatogenic damage. Furthermore, it appears that seminiferous tubule and Leydig cell functions are equally affected in this syndrome.

Ample therapeutic modalities have been used in idiopathic oligospermia. Data are presented regarding the success rate of treatment with the antiestrogens clomiphene citrate (Clomid®) and tamoxifen (Nolvadex®) of patients with idiopathic oligospermia. Interpretation of the results is often hampered by lack of agreement as to what constitutes a positive response to therapy. Furthermore, selection criteria of patients are not always consistent.

In chapter I the aim of the studies in the present thesis has been delineated.

From the data in chapter II it appears that in men with idiopathic oligospermia there is a state of compensated Leydig cell failure as plasma testosterone levels are normal but only at the cost of supranormal gonadotropin concentrations. Under basal circumstances no accumulation of the major precursor of testosterone, 17α-hydroxyprogesterone (17-OHP) occurred. During LH-RH stimulation, however, the 17-OHP/testosterone ratio significantly increased, reflecting the presence of

a 17,20-lyase block. In the oligospermic patients the rise in plasma testosterone 60 to 180 minutes after the start of the infusion was only half the increase observed in the control subjects, who did not show a 17,20-lyase block. Furthermore, in the oligospermic men the early testosterone rise was completely absent, in contrast to the finding in the eupermic men who showed a clear testosterone response 15 minutes after the start of exogenous LH-RH stimulation.
The data illustrate that in idiopathic oligospermia Leydig cell function is anomalous. Chronic desensitization by supranormal gonadotropins and/or locally secreted LH-RH-like peptides, due to seminiferous tubule damage, leading to depletion of rapidly releasable testosterone stores and enzymatic impairment, may be one of the causes of the Leydig cell dysfunction.

In chapter III the effects of lower (5 and 10 mg once daily) and higher doses (10 mg twice daily) of the competitive estrogen receptor antagonist tamoxifen on circulatory hormone levels and sperm parameters in idiopathic oligospermia are compared. The study was further designed to assess the predictary value - if any - of basal and LH-RH stimulated gonadotropin and steroid levels on spermatogenesis one month after starting tamoxifen therapy. From the data in chapter III it is clear that lower and higher doses of tamoxifen administered for about half a year are equally effective in improving total sperm counts and sperm density, without affecting motility or morphology. Sperm density normalized in one patient of either group. The concordance in stimulating effect of both the lower and higher doses of tamoxifen could already be presaged from the similarity in basal and LH-RH stimulated serum gonadotropin and steroid responses and in the testosterone/estradiol (T/E_2) ratio one month after the start of treatment.

The relatively small percentage of patients achieving normalization of the total sperm counts and density pleads for further search for more effective selection of patients and more effective treatment modalities.

In patients with idiopathic oligospermia raised intratesticular estrogen levels have been postulated as the cause of the gonadal dysfunction. It was hypothesized that these increased estrogen levels may

be the cause of the anomalous Leydig cell response to LH-RH infusion described in chapter II and that lowering endogenous estrogens by chronic administration of the aromatase inhibitor Δ^1-testolactone could correct the anomaly. The results were rather disappointing. Indeed Δ^1-testolactone administration (500 mg twice daily) for 1 month led to a 30% decrease in circulating estradiol (E_2) levels with enhancement of the FSH, 17-OHP and less T secretion and almost doubling of the T/E_2 ratio. However, both under basal and stimulated conditions, an aggravation of the LH-RH induced 17,20-lyase block occurred, in stead of the expected relieve. Furthermore, Δ^1-Testolactone completely deleted the late testosterone response to LH-RH infusion, whereas the lack of the early testosterone response in the oligospermic men persisted. Together the data may suggest that estrogens do not play a significant role in the genesis of the Leydig cell defect in oligospermia. However, the occurrence of a similar aggravation of the 17,20-lyase block in gonadotropin stimulated eugonadal men, administered Δ^1-testolactone for 1 week points to a dual action of Δ^1-testolactone i.e. inhibition of aromatase and simultaneously of 17,20-lyase. This latter effect may partially obscure the beneficial effect of lowering E_2 on testicular steroidogenesis (chapter IV).

Increased intratubular estrogen levels and/or an inordinate sensitivity of the germinal epithelium to their inhibiting effect have been postulated as the cause of the defective spermatogenesis in patients with idiopathic oligospermia. Therefore, it was considered important to investigate the effect of sustained lowering of the endogenous estrogen levels by chronic administration (6 months) of the aromatase inhibitor Δ^1-testolactone (500 mg twice daily) on endocrinological and sperm parameters in these patients. Δ^1-Testolactone administration for 6 months to 9 patients with idiopathic oligospermia lowered the levels of estradiol and thereby sex hormone binding globuline by about 30%, temporarily increased serum FSH levels and almost doubled the T/E_2 ratio (in contrast with tamoxifen which left the T/E_2 ratio unchanged) and the free testosterone index. During Δ^1-testolactone treatment the mean sperm density and total sperm count almost doubled and normospermia was achieved in 4 patients.
Although the data point to a pivotal role of estrogens in the patho-

genesis of the spermatogenic lesion in some patients with idiopathic oligospermia, the lack of a beneficial effect of estrogen lowering in others points to a multicausal nature of the disease entity (chapter V).

In chapter VI data from the previous chapters have been integrated. The need for a more profound insight in the relationship between Leydig cells, Sertoli cells and germ cells also in man is stressed. Hitherto such knowledge is almost only available from studies in rats in which a close interaction between the three testicular compartments has been demonstrated. Merely extrapolating these data to man is not possible because there are many differences between both species in steroid biosynthesis and spermatogenesis. Progress in the knowledge of the pathophysiology of idiopathic oligospermia will ultimately lead to more effective selection criteria and to more effective treatment modalities.

Verminderde vruchtbaarheid van de man is een veel voorkomende oorzaak van ongewilde kinderloosheid. Wanneer het aantal spermatozoën onder de gestelde norm valt, wordt geconcludeerd dat er sprake is van oligozoospermie. Een aantal oorzaken van oligozoospermie is bekend en vast te stellen. Vaak is dit echter niet mogelijk en wordt de verlegenheidsdiagnose idiopathische oligozoospermie gesteld.
De in dit proefschrift gepresenteerde studies hebben alle betrekking op patienten met idiopathische oligozoospermie.

In hoofdstuk I wordt oligozoospermie gedefinieerd volgens het criterium van MacLeod: minder dan 20 x 10^6 spermatozoën/ml. Alle in dit proefschrift bestudeerde mannen voldoen aan deze definitie. Tevens is er bij hen sprake van verminderde beweeglijkheid van de spermatozoën (asthenozoospermie) en een verhoogd aantal abnormaal gevormde spermatozoën (teratozoospermie) zoals meestal het geval is bij idiopathische oligozoospermie.
De verschillende oorzaken van oligozoospermie en de criteria voor het stellen van de diagnose zijn kort samengevat. Endocrinologische aspecten worden behandeld en argumenten worden aangevoerd welke de overtuiging steunen dat idiopathische oligozoospermie zowel een aandoening van de tubulus seminiferus als van de Leydig cel is. In een uitvoerig literatuuroverzicht worden de resultaten van de behandeling van patienten met idiopathische oligozoospermie met anti-oestrogenen samengevat. De interpretatie van de gepubliceerde resultaten van behandeling met respectievelijk clomipheen-citraat (Clomid®) en tamoxifen (Nolvadex®) wordt bemoeilijkt door het ontbreken van uniformiteit in de gebezigde selectiecriteria. Ook is het begrip 'significante verbetering' niet altijd nauwkeurig gedefinieerd.
Tenslotte worden de doelstellingen van de in dit proefschrift gerapporteerde studies nader uiteengezet.

In hoofdstuk II wordt nagegaan welke hormonale verschillen er onder basale omstandigheden en tijdens stimulatie met luteinizing hormone-releasing hormone (LH-RH) bestaan tussen groepen van mannen met eu-spermie en idiopathische oligozoospermie. De gevonden verschillen

steunen de opvatting dat er sprake is van een afwijkende functie van de Leydigcel bij patienten met idiopathische oligozoospermie. Onder basale omstandigheden is het plasma testosterongehalte weliswaar normaal, maar hiervoor is een sterkere LH-stimulatie nodig ('compensated Leydig cell failure'). Tijdens toediening van LH-RH stijgt de serum testosteron spiegel bij deze oligozoosperme mannen significant minder dan bij proefpersonen met euspermie. Bovendien vindt accumulatie plaats van zijn belangrijkste voorloper, het 17-hydroxyprogesteron (17-OHP). Dit wijst op een remming van het sleutelenzym 17,20-lyase. Opmerkelijk is bovendien dat bij de mannen met oligozoospermie de initiële stijging van de testosteronspiegel ontbreekt, welke wel wordt gezien bij mannen met euspermie. Dit afwijkende reactiepatroon van de Leydigcel bij idiopathische oligospermie kan een gevolg zijn van chronische desensitizering van de Leydigcel door verhoogde gonadotrofinen of door LH-RH-achtige peptiden afkomstig uit het afwijkend kiemcelepitheel ter plaatse.

In hoofdstuk III wordt het effect bestudeerd van langdurige toediening van lagere (5 en 10 mg, 1 x daags) en hogere (10 mg, 2 x daags) doseringen van de competitieve oestrogeen-receptorantagonist tamoxifen (Nolvadex®) op de serum spiegels van gonadotrofinen, testosteron en oestradiol en de kwantitatieve en kwalitatieve spermaparameters. Tevens was een doel van de studie na te gaan in hoeverre een LH-RH-stimulatietest één maand na begin van de behandeling, voorspellende waarde heeft voor de beoogde verbetering van de spermatogenese. Dit bleek niet het geval te zijn. Bovendien bleken genoemde doseringen van tamoxifen na 4 weken toediening een vergelijkbaar effect te hebben op de basale en door LH-RH gestimuleerde spiegels van gonadotrofinen en testosteron en de ratio testosteron/oestradiol (T/E_2). De basale waarden van gonadotrofinen en oestradiol en de T/E_2 ratio waren na 4 weken tamoxifen niet significant veranderd. De LH respons op LH-RH en basale testosteron waarde waren echter significant toegenomen. Ook lieten de verschillende tamoxifendoseringen geen verschil in effect zien op de spermatozoëndichtheid en het totale aantal spermatozoën per ejaculaat. Slechts bij 1 patient in beide behandelingsgroepen werd normalisering van beide parameters verkregen. Het percentage abnormale spermatozoën en hun beweeglijkheid veranderden niet. Deze teleurstellende resultaten pleiten voor betere selectiecriteria voor behandeling van patienten met oligo-

zoospermie met antioestrogenen en meer nog voor het zoeken naar andere meer effectieve behandelingsmethoden.

Bij idiopathische oligozoospermie wordt verondersteld dat de gonadale dysfunctie berust op een te hoge oestrogeenconcentratie in de tubuli seminiferi. Tevens zou een te hoge interstitiële oestrogeenconcentratie het afwijkende reactiepatroon van de Leydigcel op toediening van LH-RH kunnen verklaren. Om deze hypothese te toetsen werd in hoofdstuk IV het effect van toediening van de aromatase-remmer Δ^1-testolacton (Teslac®) op genoemd reactiepatroon onderzocht. De resultaten waren verrassend. Inderdaad verlaagde Δ^1-testolacton de serum oestradiolspiegel binnen 1 maand met 30%. De serumspiegels van FSH, 17-OHP en in mindere mate testosteron, stegen en de T/E_2 ratio verdubbelde. Echter tegen de verwachting in trad zowel onder basale omstandigheden als tijdens toediening van LH-RH een verergering van de 17,20-lyase remming op. Bovendien deed toediening van de aromatase-remmer de door LH-RH gestimuleerde late stijging van testosteron teniet, terwijl dit niet het geval was met 17-OHP. Een initiële stijging van testosteron zoals bij eugonadale controle personen werd niet waargenomen. Tezamen suggereren deze gegevens dat oestrogenen niet zo'n belangrijke rol spelen bij de genese van de gonadale dysfunctie als wel wordt aangenomen. Opmerkelijk is, dat vergelijkbare bevindingen ook werden verkregen bij eugonadale mannen, die kortdurend Δ^1-testolacton kregen toegediend. Aangenomen moet dan ook worden dat Δ^1-testolacton een tweeledig effect uitoefent. Het bewerkstelligt de beoogde remming van de omzetting van androgenen naar oestrogenen door beinvloeding van het enzym aromatase, maar tevens een ongewenste remming van het sleutelenzym 17,20-lyase. Deze laatste werking doet minstens gedeeltelijk teniet het gunstige effect van verlaging van intratesticulaire oestrogeenconcentratie op de synthese van steroiden door de Leydigcel.

Een verhoogde intratubulaire concentratie van oestrogenen of een verhoogde gevoeligheid van het kiemepitheel voor oestrogenen zou een causale factor kunnen zijn bij het ontstaan van oligozoospermie. Dit was de reden om patienten met idiopathische oligozoospermie gedurende 6 maanden te behandelen met de aromatase-remmer Δ^1-testolacton. De resultaten hievan zijn in hoofdstuk V beschreven. Het voordeel van toedie-

ning van Δ^1-testolacton boven tamoxifen blijkt uit de blijvende daling van de spiegels van oestrogenen en het testosteron-bindend globuline tijdens Δ^1-testolacton en de daarme gepaard gaande tijdelijke stijging van de serum FSH-spiegel en verdubbeling van zowel de T/E_2 ratio als vrije testosteron index. Bij 4 van de 9 met Δ^1-testolacton behandelde patienten vond tijdens deze behandeling normalisering plaats van de spermatozoëndichtheid. Ook daalde het percentage abnormale spermatozoën. Omdat bij de overige patienten slechts een geringe of geen verbetering van deze parameters optrad, moet echter worden geconcludeerd, dat een verhoogde intratubulaire oestrogeenconcentratie of een verhoogde gevoeligheid van het kiemepitheel minder vaak verantwoordelijk is voor de afwijkende spermatogenese dan gemeenlijk wordt aangenomen.

In hoofdstuk VI worden de gegevens uit de hoofdstukken I t/m V geïntegreerd. Bovendien wordt gewezen op de noodzaak om de kennis te vergroten van de interactie tussen Leydigcellen, Sertolicellen en kiemcellen bij de mens. Thans bestaat dergelijke kennis alleen uit experimenten verricht bij proefdieren, vooral de rat. Extrapolatie van deze kennis naar de mens is niet zonder meer mogelijk, gezien de vele verschillen welke bestaan tussen beide species wat betreft de synthese van steroiden en de spermatogenese.
Tenslotte worden mogelijkheden aangegeven waardoor het inzicht in de pathogenese van idiopathische oligozoospermie kan worden verdiept. De hoop is dat dit resulteert in betere selectiecriteria en betere resultaten van behandeling.

Dit proefschrift is het resultaat van vruchtbare samenwerking in het onderzoekscluster 'Endocriene Regelmechanismen'.
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CURRICULUM VITAE

20.5.1939 : Geboren te Princenhage-Breda

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1966 : Doktoraalexamen Geneeskunde, Rijksuniversiteit te Utrecht

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1968 : Drie maanden waarneming in huisartsen praktijk

1968 - 1974 : Opleiding tot vrouwenarts in de kliniek voor Obstetrie en Gynaecologie van het St. Radboudziekenhuis te Nijmegen. Opleider: Prof.dr J.L. Mastboom

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1978 - 1981 : Lid van het Directoraat Klinieken van het Sint Radboudziekenhuis.
Vanaf 1973 werkzaam als staflid van de kliniek voor Obstetrie en Gynaecologie van het St. Radboudziekenhuis te Nijmegen.
Sinds 1974 chef de clinique gynaecologie en verantwoordelijk voor de andrologie

STELLINGEN

behorend bij het proefschrift

Idiopathic Oligospermia

J.M.J. Dony

STELLINGEN

I

Bij idiopathische oligozoospermie is de functie van de Leydigcel gestoord.

II

De selectiecriteria voor behandeling van patiënten met idiopathische oligozoospermie met tamoxifen (Nolvadex®) of Δ^1-testolactone (Teslac®) zijn voor verbetering vatbaar.

III

De 17,20-lyase remming tijdens LH-RH stimulatie bij mannen met idiopathische oligozoospermie wordt niet ongedaan gemaakt door inname van 1 gram Δ^1-testolacton (Teslac®) gedurende 4 voorafgaande weken.

IV

De opvatting, dat de actieve uitdrijvingsfase dient te beginnen zodra volledige ontsluiting is bereikt, getuigt van obstetrische onervarenheid.

V

De behandeling van vaginisme met pelotte-therapie faalt zelden, indien anamnestisch is gebleken dat er sprake is van een positieve sexuele attitude en van voldoende motivatie.

VI

De kans op mislukken van een laparoscopische sterilisatie door 'doctor's failure' wordt gereduceerd indien de uterus met een hulkaklem in optimale anteflexie wordt gebracht.

VII

'Pull up' operaties, die zowel tot doel hebben de blaashals omhoog te brengen als gelijktijdig een cystocele te corrigeren, predisponeren tot het ontstaan van een enterocele.

VIII

Sacraal gerichte fixatie van de blind-eindigende vaginatop biedt minder kans op topprolaps dan ventrale fixatie.

IX

De opvatting, dat de conceptiekans wordt vergroot door een extra lange abstinentieperiode, zo diep mogelijke intravaginale ejaculatie en volledigheid van de laatste spermafractie, is onjuist.

X

Semenplasma is een slecht medium voor spermatozoën.

XI

Bij een uterus in mobiele retroversie, die zich niet zonder meer in anteflexie laat brengen, dient de mogelijkheid van een myoom in de uterusachterwand overwogen te worden.

XII

De bijsluitertekst van verpakte geneesmiddelen in zijn huidige vorm geeft inadequate informatie aan de gebruiker.

XIII

De profielschets van de universitaire (hoofd)docent biedt geen waarborg voor de benoeming van echte docenten.

XIV

Zowel in de tandheelkunde als in de gynaecologie gaat sonderen met risico's gepaard.

XV

Bij de beoordeling van het testisvolume van een ander, neigt de minder ervaren onderzoeker ertoe zijn eigen testisvolume als 'modaal' te beschouwen en daaraan te refereren.

XVI

De opgeslagen achterste arm bij de mediane episiotomie maakt een van beiden 'misplaatst'.

XVII

Zegswijzen als 'doe niet zo gauche', 'twee linker handen' en de betekenis van dexterity zijn discriminerend voor minstens sommige linkshandigen.

XVIII

Te weinig gynaecologen zijn tevens androloog en te weinig andrologen zijn tevens gynaecoloog.