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WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 514

AGENTS STIMULATING GONADAL FUNCTION IN THE HUMAN

Report of a WHO Scientific Group

WORLD HEALTH ORGANIZATION
GENEVA
1973

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PRINTED IN FRANCE

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GONADAL FUNCTION IN THE HUMAN

Geneva, 28 August - 1 September 1972

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AGENTS STIMULATING GONADAL FUNCTION IN THE HUMAN

Report of a WHO Scientific Group

A WHO Scientific Group on Agents Stimulating Gonadal Function in the Human met in Geneva from 28 August to 1 September 1972. The meeting was opened by Dr A. Zahra, Director, Division of Family Health, who welcomed the members on behalf of the Director-General.

1. INTRODUCTION

On numerous occasions WHO scientific groups, the Advisory Committee for Medical Research, and World Health Assemblies have recommended that WHO periodically evaluate advances in fertility regulation.

The primary objectives of the present meeting were to review available information and current research on agents used for the stimulation of gonadal function, particularly for the treatment of infertility. Attention was focused on clinical research to better define the selection of patients, the appropriate agents, and the management and monitoring of patients under therapy. In order to obtain guidelines for the clinical assessment of agents to promote human fertility the Scientific Group critically reviewed their accumulated experience and the results of other investigators. From this information a consensus was reached on the problems of managing infertile patients treated with agents stimulating gonadal function. It was also felt that further research and information were necessary to narrow the gaps in knowledge in this field.

The assessment of the clinical effectiveness of agents stimulating gonadal function is difficult since few statistically well designed studies have been conducted. The problems of interpreting many reports are highlighted by a study in which placebo therapy in women suffering from amenorrhoea resulted in a pregnancy rate of about 20%. In this report, therefore, emphasis is placed on agents with well established effectiveness.

Furthermore, the Group wishes to emphasize that the conclusions reached in this report are based on studies carried out in a relatively small number of major centres in Europe, Israel, Japan, North America, and Oceania. There is no reason to believe that these conclusions do not apply equally well to other population groups; however, this statement can be confirmed only by properly conducted clinical research in those groups. The quantitative data given in the report, relating to the measurement of endocrine function, should also be interpreted with this reservation in mind.

2. FERTILITY PROMOTION IN THE FEMALE

2.1 Pertinent reproductive processes

Ovulation is the result of an integrated action between the hypothalamus, the anterior pituitary, and the ovaries. The harmonious balance between the gonadotrophic hormones and ovarian steroids leads to an orderly ovulatory sequence. The gonadotrophin-releasing hormones stimulate the synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which results in ovarian follicular growth and maturation. As a consequence, estrogens and other ovarian hormones are produced that appear to influence the hypothalamo-pituitary axis to induce an LH and FSH surge responsible for ovulation. Failure to ovulate may be the result of dysfunction at any level of this complex system, including higher centres in the brain, the hypothalamo-pituitary-ovarian axis, and the steroid feedback mechanism.

2.2 Fertility promoting agents

Absent or altered gonadotrophin secretion in the presence of responsive ovaries can be remedied. The administration of FSH and LH from human sources in correct amounts, sequence, and duration can achieve an ovulatory response.

In attempting to stimulate ovarian function, numerous techniques have been used. This report covers therapy with gonadotrophins, clomifene citrate, cyclofenil, estrogens, progesterone, synthetic progestational agents, and estrogen-progestogen combinations. Gonadotrophin-releasing hormones have also been considered as potential gonadal stimulating agents.

If pregnancy is the final goal, the therapeutic use of agents to stimulate gonadal function is justified only if the anatomical integrity of the utero-tubal tract is first ascertained. Proper attention should be paid to the fertility potential of the male partner.

2.3 Selection of patients

The Scientific Group recommended the following classification¹ of patients as a guide to therapy. The Group realized that in the future the use of gonadotrophin-releasing hormone preparations may permit the assessment of pituitary responsiveness, which would help to differentiate ovarian dysfunction originating in the pituitary from that originating in the hypothalamus and/or in the central nervous system. This may in turn lead to some modification of the classification suggested.

Group I — Women with amenorrhoea and with little or no evidence of endogenous estrogen activity, including patients with (a) hypogonadotrophic ovarian failure, (b) complete or partial hypopituitarism, or (c) pituitary-hypothalamic dysfunction.

Patients of this group may be suffering from either primary or secondary amenorrhoea. Bioassays or immunoassays reveal that serum and urinary gonadotrophins are low or unmeasurable. Whenever possible specific assays should be made for FSH and LH rather than a determination of total gonadotrophins. Estrogen levels should be low, as evidenced either by direct assay (urinary estrogens <10 µg/24 h, plasma estrogens <100 pg/ml) or by indirect clinical methods such as cytological examination of vaginal smears and/or urinary sediment (parabasal cells), examination of the cervical mucus (absence of ferning pattern), and endometrial biopsy (atrophic changes). Furthermore, plasma progesterone levels should be less than 1.0 ng/ml and plasma hydroxyprogesterone values should be less than 0.2 ng/ml. Finally the oral or parenteral administration of a suitable progestational agent should not produce withdrawal bleeding.

For the time being, the treatment of choice for patients of this group is with gonadotrophins.

Group II — Women with a variety of menstrual cycle disturbances (including amenorrhoea) who exhibit distinct endogenous estrogen activity (urinary estrogens usually <10 µg/24 h), whose urinary and serum gonadotrophins are in the normal range and fluctuating, and who may also have fairly regular spontaneous menstrual bleedings (i.e., less than 35 days apart) but without ovulation.

For these patients, drugs stimulating endogenous gonadotrophin release, e.g., clomifene, are recommended. Patients who do not respond to repeated courses of such agents may be considered for gonadotrophin therapy.

Subjects with amenorrhoea following the use of oral hormonal contraceptives ("post-pill amenorrhoea") and patients with galactorrhoea associated with amenorrhoea usually belong to Group II. However,

¹ This classification is a modification of one suggested by the WHO International Reference Centre for Fertility Promoting Agents in 1971.

on the basis of laboratory and indirect clinical findings some may be classified as Group I. Irrespective of the group to which they belong, the preferred treatment for such patients is with agents stimulating the release of endogenous gonadotrophins, e.g., clomifene. Patients who do not respond to repeated courses of such agents may be considered for gonadotrophin therapy.

Group III — Women with primary ovarian failure associated with low endogenous estrogen activity and pathologically elevated serum and urinary gonadotrophins.

For this group, agents stimulating gonadal function are, as a rule, ineffective and it is felt that there is little, if any, justification for treating patients exhibiting primary ovarian failure with such agents.

3. GONADOTROPHIC PREPARATIONS

Since gonadotrophic preparations derived from animal sources produce neutralizing antibodies, gonadotrophins prepared from human sources are preferred in the modern treatment of anovulation.

Human gonadotrophins are prepared either from pituitary glands obtained at autopsy (human pituitary gonadotrophin, HPG) or from the urine of postmenopausal women (human menopausal gonadotrophin, HMG). Each type of preparation exhibits both FSH and LH activity. Most investigators consider that HMG and HPG are equally effective in inducing ovulation.

Initially, owing to the lack of appropriate standard preparations, there was confusion about how much FSH and LH was present in any given batch of HPG or HMG. This problem has been solved by defining the FSH and LH activities of each preparation in terms of the second International Reference Preparation of Human Menopausal Gonadotrophins. To achieve this it has been necessary to use specific bioassays, such as the Steelman Pohley technique for estimating FSH potency and either the ovarian ascorbic acid depletion method or the ventral prostate weight assay for estimating LH potency. From a therapeutic point of view, the present designation of FSH and LH in terms of international units per milligram or per ampoule is satisfactory for treating ovarian and testicular disorders and for comparing the therapeutic regimes used by different clinicians.

The ratio of FSH to LH varies in different HMG and HPG preparations, but the available evidence indicates that preparations with ratios of 0.1-10.0 are acceptable therapeutic agents provided that a sufficient total dosage of FSH is administered to the patient.

Additional LH in the form of human chorionic gonadotrophin (HCG) or human pituitary LH (HLH) is required to induce ovulation in the female irrespective of the usual amounts of LH present in the available HMG and HPG preparations. The data available on highly purified urinary LH are insufficient to demonstrate its efficacy, but there are no theoretical reasons to suggest that this source of LH should not be effective if used properly.

3.1 Monitoring of gonadotrophin therapy

The purpose of monitoring human gonadotrophin treatment is to attempt to obtain an ovarian response that approximates as nearly as possible to the ovarian changes that take place during a normal menstrual cycle, which means that only one egg should be released from the ovaries and that a period of about 2 weeks should elapse between the day of ovulation and the menstrual bleeding. Furthermore, since knowledge about the mode of action of gonadotrophins on the human ovaries is limited, it is hoped that careful monitoring of gonadotrophin treatment will provide new information about the female reproductive processes. Therefore, it is important during each treatment with human gonadotrophins (1) to assess the optimal daily dose of HMG or HPG, (2) to assess the number of days this dose should be administered, (3) to decide when HCG or LH administration should be commenced, (4) to determine if and when ovulation took place, (5) to assess the function of the corpus luteum, and (6) to confirm conception. If this 6-fold control is carried out correctly, the chance of a successful outcome of therapy increases and the risk of side effects becomes negligible.

During the process of follicular growth the ovary secretes increasing amounts of estrogen, while the corpus luteum secretes both estrogens and progesterone. Since direct estimation of follicular ripening is practically impossible, indirect indicators have to be used for monitoring the effect of gonadotrophins on the ovary. Thus, the response of the target organs to ovarian steroids (as shown by vaginal smears, cervical mucus, and endometrial biopsy) and the levels of these steroids and their metabolites in urine or blood are used as indicators of ovarian response during gonadotrophic therapy.

In order to avoid adverse reactions (hyperstimulation) and to reduce the chance either of multiple pregnancy or of insufficient stimulation, it is important to monitor the ovarian response of the patient. Therefore it is a great advantage if the treatment is undertaken by an experienced physician in centres equipped with suitable facilities. In difficult patient-management problems it is a major handicap not to have some of the necessary laboratory procedures available.

Combinations of the following methods, which are of varying specificity, have been used to assess ovarian response to HMG or HPG administration:

- assessment of cervical reactions ("cervical score")
- determination of urinary estrogens
- measurement of ovarian size
- determination of plasma estradiol and total estrogens
- determination of plasma hydroxyprogesterone and progesterone
- determination of urinary pregnanediol
- estimation of the karyopyknotic index
- measurement of sialic acid concentration in cervical mucus
- determination of sperm penetration.

Among these the estrogen assays are most valuable because they assist the physician both in avoiding hyperstimulation and in determining the optimum time for the induction of ovulation by HCG or HLH.

A brief description of some of the above methods is given to acquaint the reader with their respective assets and limitations.

Cervical score

This is a semiquantitative estimate of 4 variables: the amount, spinnbarkeit, and ferning capacity of the cervical mucus and the appearance of the external os. Each variable is awarded a score of 0-3, according to the criteria given in the accompanying table. In patients undergoing gonadotrophin treatment a total score of 9-12 is considered to represent the optimum time for HCG administration. The cervical score has certain limitations in that it does not always reflect circulating estrogen levels.

Urinary estrogens

Daily determination of total urinary estrogens according to Brown et al. (1968)¹ has been used successfully to obtain information about the optimum daily dose of HMG or HPG, the duration of administration, and the appropriate day for HCG administration. The method allows 12 determinations in about 4 hours.

Ovarian size

A rapid increase in ovarian size may presage a hyperstimulation reaction. Unfortunately, in many patients it is difficult to assess changes in ovarian size by palpation.

¹ Brown, J.B., MacLeod, S.C., Macnaughtan, C., Smith, M.A., & Smyth, B. (1968) *J. Endocr.*, **42**, 5.

CERVICAL SCORE FOR EVALUATING ESTROGEN ACTIVITY^a

		Cervical score			
		0	1	2	3
Amount of mucus	None	Scant A small amount of mucus can be drawn from the cervical canal	Dribble A glistening drop of mucus seen in the external os; mucus easily drawn from the cervical canal	Cascade Abundant mucus pouring out of the external os (more than 400 µl)	
Spinnbarkeit	None	Slight Uninterrupted mucus thread may be drawn about a quarter of the distance between the external os and vulva	Moderate Uninterrupted mucus thread may be drawn about half the distance between the external os and vulva	Pronounced Uninterrupted mucus thread may be drawn for the whole distance between the external os and vulva (more than 8 cm)	
Ferning	None Amorphous mucus	Linear Fine linear ferning seen in a few spots, no side branching	Partial Good ferning with side branches in parts of the slide; linear ferning or amorphous mucus in other parts	Complete Full ferning of the whole preparation	
Cervix	Closed Mucosa pale pink; the external os hardly admits a thin applicator (<2 mm)	Partially open Mucosa pink; the cervical canal is easily penetrable by an applicator (2-4 mm)	Gaping Mucosa hyperaemic; the external os patulous (>4 mm)		

^a This scheme is a modified version of one recommended by the WHO International Reference Centre for Fertility Promoting Agents, in its 1971 report to the Organization.

Plasma steroid levels

Plasma levels of estradiol (and/or estrone) furnish the same type of information as do total urinary estrogens, but their measurement requires special equipment and experienced quality control. Plasma hydroxyprogesterone shows a characteristic pattern around the time of ovulation. Plasma progesterone levels give information on ovulation and corpus luteum function.

Urinary pregnanediol

The determination of this progesterone metabolite generally provides the same information as that given by plasma progesterone levels.

Karyopyknotic index

The vaginal mucosa is generally expected to reflect circulating estrogen levels. However, the estimation of the karyopyknotic index has major limitations, especially in the presence of vaginal infections and bleeding. Further-

more, similar regressive changes are induced by a decrease in estrogen levels and by a simultaneous increase in estrogen and progesterone levels.

3.2 Analysis of response to gonadotrophic stimulation

Special care should be taken to evaluate ovarian function before and during treatment. Urinary or plasma estrogen titres in combination with changes in the cervical mucus are the most helpful criteria in this regard. Increased follicular activity (as shown by urinary and/or plasma estrogens) is usually detected 3-4 days before ovulation, while in HMG- or HPG-induced cycles an increase in follicular activity is obtained about 4-6 days prior to the time when ovulation is induced. Keeping in mind certain limitations, evaluation of cervical mucus changes provides an important adjunct to estrogen determinations. The diagnosis of ovulation should be based on several observations such as an elevation in basal body temperature,¹ increased urinary pregnanediol excretion, and increased plasma progesterone levels. Early detection of pregnancy should be attempted by estimating HCG with radio-immunoassay techniques approximately 10 days after the last injection of HCG.

In order to compare the treatment results between various centres, rates of ovulation, pregnancy, fetal wastage, and adverse reactions should be carefully documented.

3.3 Treatment scheme

The treatment schedule is one of the main factors governing the results of gonadotrophic therapy. An ideal scheme of gonadotrophic treatment should eventually produce pregnancy in all suitable patients of Groups I and II while avoiding hyperstimulation. Unfortunately, such an ideal schedule has not yet been devised. This is chiefly because of the variations between patients within the same group classification and because of the variations in response in the same patient during different treatment cycles. In general, most investigators start with a relatively low daily dosage of HMG or HPG and then gradually increase the dosage according to the cervical mucus response and the results of estrogen determinations.²

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 360, p. 12.

² The following data were obtained in a collaborative study:

(a) The mean of total dose of FSH for Group I was 3000 IU, with an interpercentile range (95 %) of 1350-5850 IU. The mean of the total FSH dose for Group II was 1200 IU, with an interpercentile range (95 %) of 750-4050 IU.

(b) The effective daily dose for the majority of patients in Group I was in the range 150-225 IU, whereas for patients of Group II it was in the range 75-150 IU.

(c) The duration of treatment of Group I was about 14 days while that of group II was about 9 days.

Two types of treatment schemes have been described:

(a) an individually adjusted schedule in which the amount of gonadotrophin is determined in each cycle by the patient's ovarian response, and

(b) a fixed schedule in which a predetermined dose of gonadotrophin is administered either daily or on alternate days, the effective dose being obtained by increasing the initial predetermined dose in consecutive treatment cycles.

When the effective FSH dose is reached in either of these treatment schemes, it is continued until two or more of the following measurements are obtained:

(1) urinary estrogens: 60-100 $\mu\text{g}/24$ hours

(2) plasma estrogens: 800-1000 pg/ml

(3) plasma progesterone: less than 1 ng/ml (The reason for fixing this limit is that occasionally patients may ovulate with HMG or HPG therapy alone; thus if the progesterone levels are above 2-3 ng/ml, suggesting luteinization or ovulation, the administration of HCG or HLH is not recommended.)

(4) cervical score: 9-12

(5) plasma hydroxyprogesterone: 3-5 ng/ml.

At this stage HCG should be administered. The time factor is crucial. If HCG is given too early, ovulation might not occur; if it is postponed, and HMG or HPG therapy is continued, the danger of producing hyperstimulation is significantly increased.

Various dosage and time schedules have been employed with rather similar results. HCG is usually given 24-48 hours after the last HMG or HPG injection. The amount of HCG administered at this time varies between 5 000 and 10 000 IU. There appears to be no rationale to support the scheme in which HCG administration overlaps with HMG or HPG administration. Most investigators give HCG only once, some administer HCG on two consecutive days, while others give HCG 48 hours after the last HMG dose, followed by another injection of HCG one week later. The rationale of this last scheme is to opt for a luteal phase of 12-14 days.

When gonadotrophin-treated patients become pregnant they should be considered as having a high-risk pregnancy, particularly when a multiple pregnancy is detected.

The number of treatment cycles that result in ovulation but not in pregnancy was also considered. The Group felt that after 3-4 cycles with the effective dose, gonadotrophin therapy should be discontinued and the patient re-evaluated with respect to other factors that may cause infertility.

The Group felt that, with patients having ovarian dysfunction but not desiring a pregnancy, a complete diagnostic examination should be performed but repeated induction of ovulation should be postponed until the patients wish to conceive.

The age of the patient is another important consideration. Because the incidence of maternal morbidity and mortality and of fetal malformations increases with advancing age, the Group considered that patients about 40 years of age should fully be informed of these hazards.

3.4 Complications of gonadotrophin therapy

The complications of gonadotrophin therapy are:

- (1) the hyperstimulation syndrome
- (2) an increased incidence of multiple pregnancies
- (3) an increased fetal wastage.

The factors that may give rise to these complications include: (a) excessive HMG administration, (b) incorrect selection of patients, (c) inadequate monitoring of treatment, and (d) inappropriate HCG administration. Even with careful monitoring, however, complications may still occur. The hyperstimulation syndrome is a particularly important one and may require special therapeutic measures. To improve its management the following classification is proposed.

Grade I: These patients have variable ovarian enlargement, sometimes associated with small cysts. Laboratory findings include urinary estrogen levels of over 150 $\mu\text{g}/24$ hours and pregnanediol excretion titres of over 10 mg/24 hours. Treatment is not necessary, but the patients should be advised to report immediately to their physician if additional symptoms appear.

Grade II: Patients in this category have additional symptoms of a variable nature. They include abdominal distension, nausea, vomiting, and diarrhoea. Careful medical observation is required and appropriate symptomatic treatment is indicated.

Grade III: These patients are characterized by having large ovarian cysts, ascites, and sometimes hydrothorax. Haemoconcentration with increased blood viscosity and coagulation abnormalities may appear. Hospitalization and prompt treatment are indicated. Therapy should be directed towards correcting the altered fluid and electrolyte balance. This is accomplished by the administration of 5% glucose in water (without sodium chloride) in conjunction with sodium-mobilizing diuretics (e.g.,

furosemide) to improve urinary output. The use of plasma expanders may also be indicated. Anticoagulant therapy should not be used on a routine basis. Removal of ascitic and/or pleural fluid is indicated only when pulmonary function is impaired or in cases of extreme discomfort.

The pathogenesis of severe hyperstimulation is not entirely clear but it appears that a fundamental factor is excessive estrogen production. This phenomenon leads to capillary damage, salt retention, and increased platelet adhesiveness. When haemoconcentration occurs, aldosterone production increases.

It should be noted that abdominal or pelvic examination should be cautiously gentle in these patients in order to avoid rupturing the ovarian cysts. Although laparotomy to stop bleeding from a ruptured ovarian cyst may occasionally become necessary, ovariectomy should not be carried out.

3.5 Prevention of hyperstimulation syndrome

Hyperstimulation is not always easy to predict. Careful monitoring of treatment by estrogen determination is the best insurance against this problem. When the slope of estrogen excretion becomes steep, the physician should be cautious about the current dosage of HPG and should even consider stopping therapy altogether. If the urinary estrogen level is above 120 $\mu\text{g}/24$ hours, HPG should be withheld. Although these figures are presented as guidelines, it should be noted that high levels of estrogens are not always associated with the development of hyperstimulation.

Multiple pregnancies and fetal wastage are not as closely related to excessive gonadotrophin administration as is hyperstimulation. Further studies are needed to clarify the etiological factors involved in these two complications of gonadotrophin therapy.

In summary, it may be concluded that for the time being gonadotrophin therapy is the most efficient tool in the treatment of infertility due to anovulation. However, one must keep in mind the risk of multiple pregnancy (about 20 %), spontaneous abortion (19 %), and adverse reactions (about 1 %).

4. CLOMIFENE

Clomifene citrate,¹ a synthetic preparation, is an analogue of chlorotrianisene and is related to the potent estrogen diethylstilbestrol. It is available in the racemic form, which has a 1:1 ratio of the *cis* and *trans*

¹ 2-[*p*-(2-chloro-1,2-diphenylvinyl)phenoxy]triethylamine citrate.

forms of the compound. On a weight basis, the *cis* form is more potent than the racemic form in stimulating LH and FSH secretion, and the racemic form is more potent than the *trans* form.

4.1 Mode of action

The mechanism of action of clomifene is not clear. Many investigators consider that its anti-estrogenic property is fundamental to its action, but this view is not shared by others, and more work is necessary to clarify this problem. Nevertheless, clomifene administration to women and men with an intact hypothalamo-pituitary system causes an increase in serum and urinary LH and FSH. Thus, in addition to its use for inducing ovulation, clomifene may be used as a test for hypothalamo-pituitary function.

4.2 Selection of patients

In general, patients of Group II (section 2.3) respond well to the administration of clomifene. Patients with "post-pill amenorrhoea", whether in Group II or Group I, are also candidates for this form of therapy. The remaining patients in Group I either do not respond or respond poorly to clomifene treatment.

4.3 Treatment schemes

Doses of 50-100 mg per day are administered for 5-10 days. This treatment is usually started 5-10 days after the onset of a spontaneous or induced bleeding period. Daily doses of 200 mg should be administered with extreme caution, especially to women having polycystic ovarian disease whether or not hirsutism is present. The Scientific Group suggested that, if no pregnancy ensues after some 3-6 courses of clomifene, an additional 3-4 treatment courses should be administered in conjunction with an injection of HCG 3-7 days after the last clomifene dose. If patients still fail to conceive after these treatment schemes, HMG or HPG therapy should be considered.

Clomifene therapy is also useful in cases in which pregnancy is not the ultimate goal; it can help some women who have anovulatory cycles associated with irregular menses to achieve better menstrual regulation.

4.4 Monitoring of clomifene therapy

The progress of clomifene therapy can be monitored by observing the changes in such variables as cervical mucus, basal body temperature,

plasma progesterone titres, and urinary pregnanediol excretion levels. Since the hyperstimulation syndrome occurs less frequently with clomifene than with gonadotrophin therapy, the determination of daily urinary estrogen levels is less critical. Similarly, repeated pelvic examinations are not mandatory. Indeed, many investigators perform only one additional pelvic examination (after the immediate pretreatment period) about 2 weeks after the last dose of clomifene. If enlarged ovaries are detected at this time, the spacing of subsequent treatment cycles should be reconsidered. In any case, these patients require a pelvic examination before clomifene therapy is reinstated.

4.5 Treatment results

The administration of clomifene in doses of 50-100 mg for 5-10 days results in ovulation in 50-70 % of patients, with an overall pregnancy rate ranging from 25 % to 35 %. The multiple pregnancy rate is about 6 %, which is significantly lower than with gonadotrophin therapy (20 %). The fetal wastage (approximately 20 %) is similar to that encountered with gonadotrophin therapy.

The Scientific Group felt that there is a discrepancy between ovulation and pregnancy rates. There may be several reasons to account for this lack of correlation:

(a) ovulatory response cannot always be judged from basal body temperature, endometrial biopsy, and pregnanediol values

(b) the cervical mucus may not be favourable for the reception of sperm at the time of ovulation

(c) FSH and LH levels may be less than optimal for adequate corpus luteum function, orderly endometrial development, and ovum implantation

(d) the number of treatment trials in patients who ovulate on clomifene may be inadequate.

4.6 Side effects

As indicated before, the hyperstimulation syndrome occurs less frequently with clomifene than with gonadotrophin therapy.

Additional adverse reactions are rare, but hot flushes and changes in retinal physiology have been recorded. The latter results in blurred vision, scotoma, and sometimes decreased visual acuity. These side effects clear promptly after the cessation of clomifene administration.

5. CYCLOFENIL

5.1 Mode of action

Available evidence seems to suggest that cyclofenil¹ is capable of modifying the synthesis and secretion of FSH and LH. In contrast to clomifene citrate, cyclofenil does not exert a peripheral anti-estrogenic effect. When cyclofenil was administered to anovulatory women, an increase in urinary LH excretion levels was observed in most of the patients regardless of whether ovulation was induced or not. In those patients who appeared to ovulate, the increase in urinary LH titres occurred at about the time of presumed ovulation, but increases in urinary FSH were not consistent. The Scientific Group concluded that the mechanism of action of cyclofenil is incompletely understood.

5.2 Dosage and treatment scheme

Most investigators have used cyclofenil in doses of 500-800 mg daily for 5-10 days cyclically whereas others have used continuous therapy for many months at a daily dose of 200-400 mg.

5.3 Treatment results

The ovulation rate following treatment has been reported to be between 32% and 64% and the pregnancy rate between 4% and 22%. A collaborative study reported that of 197 pregnant women 3 gave birth to twins. This incidence is lower than that observed with gonadotrophin therapy (20%) and with clomifene treatment (6%).

The Scientific Group felt that from the available data the overall efficacy of cyclofenil cannot yet be assessed.

5.4 Side effects

In one cooperative study of 2 413 treatment cycles, a low frequency of side effects was observed. They comprised:

- (a) ovarian enlargements, 0.17%
- (b) hot flushes, 0.5%
- (c) lower abdominal pain, 0.9%
- (d) nausea and/or vomiting 0.87%.

¹ 4,4'-(cyclohexylidene)methylene)diphenol diacetate ester.

None of these side effects were of Grade II or III (section 3.5). Furthermore, these adverse reactions cleared promptly after cessation of treatment.

6. EPIMESTROL

Reports are available on the use of epimestrol¹ in inducing ovulation. The dose administered was usually 5 mg daily for 10 days from the 5th day of the cycle. The mechanisms of action of this compound are incompletely understood, and the Group felt that the available information does not permit an assessment of its merits.

7. OTHER AGENTS

Progesterone, synthetic progestational agents, estrogens, and estrogen-progestogen combinations have also been used to stimulate ovarian function in a variety of menstrual disorders including secondary amenorrhoea, functional sterility, and corpus luteum insufficiency. It is difficult to evaluate the results reported, since in most instances neither the selection of patients nor the administration of the various agents was uniform enough to permit generalizations.

It appears, however, that on discontinuation of the above therapy some patients in Group II start ovulatory cycles. The Scientific Group agreed that a major difficulty of this type of therapy is the selection of patients who are likely to respond favourably.

8. GONADOTROPHIN-RELEASING HORMONES

A number of investigators have been able to demonstrate the presence of gonadotrophin-releasing hormones (Gn-RH)² in hypothalamic extracts from several animal species. Details of the isolation, purification, and biological effects of releasing factors or hormones are given in the review articles in list 5 of the Annex. Porcine and ovine Gn-RH has been identified as a decapeptide with the sequence of (pyro)-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂. This synthetic decapeptide (DP), which has been prepared in various laboratories, possesses physicochemical properties identical to those of natural Gn-RH. Although several lines of investigation point to the existence of a separate LH-releasing hormone (LH-RH) and

¹ 3-methoxyestra-1,3,5(10)-triene-16 α ,17 α -diol.

² This expression is used to denote activities referred to as LH-RH, LH/FSH-RH, or FSH-RH.

FSH-releasing hormone (FSH-RH), it has recently been demonstrated that pure DP induces the release of both LH and FSH in various animal species and in humans. The biological and some physicochemical properties of the Gn-RH isolated from human hypothalamic extracts are similar to those of porcine Gn-RH. The structure of Gn-RH in species other than the pig and the sheep has not yet been elucidated. On the basis of these findings and the demonstration of biological activity of porcine Gn-RH in human beings, it has been postulated that the structure of human Gn-RH is similar to that of DP.

8.1 Clinical effects of natural or synthetic decapeptide

Clinical experience with Gn-RH has been based on the administration of synthetic DP and of natural material extracted from porcine, ovine, and bovine hypothalami. Rapid intravenous administration of 10-150 μ g of DP causes, in normal men and women, a significant release of pituitary LH and FSH. Maximum serum LH values are obtained 20-30 minutes after injection, whereas FSH serum levels rise more slowly, reach lower titres and have broader peaks. Subcutaneous and intramuscular injection of DP results in a more prolonged elevation of LH and FSH. No side effects have been observed after either intravenous or intramuscular DP administration.

In general, clinical experience has been limited to the use of natural and synthetic DP in diagnostic tests to evaluate pituitary gonadotrophin reserve in various hypothalamo-pituitary disorders. The administration of DP was shown to induce ovarian follicular maturation and ovulation in women with ovarian insufficiency due to hypothalamic dysfunction. Although preliminary reports are encouraging, the Group felt that for the time being it is not possible to make definite recommendations on the therapeutic use of Gn-RH, but it was agreed that it is a useful diagnostic agent for the assessment of the functional integrity of the pituitary.

9. HYPOTHALAMO-PITUITARY-GONADAL PROCESS IN THE MALE

The endocrine system is controlled, as a rule, by a feedback mechanism. Regulation of testicular function appears to be no exception, although the details of control are at present incompletely understood.

The testes serve two roles. The first involves the synthesis and secretion of testosterone by the Leydig interstitial cells, which are found interspersed in groups between the seminiferous tubules. The second function involves spermatogenesis, which is the development and maturation

of the germ cells in the epithelium of the seminiferous tubules. From the spermatogonia, which line the basement membrane of the tubules, to the development of spermatozoa, spermatogenesis is an orderly and active process in the normal adult male. Using tritiated thymidine and autoradiographic techniques, research workers have estimated the time required for human spermatogenesis to be about 74 days.

Both aspects of testicular function are under the control of gonadotrophic hormones secreted by the anterior pituitary gland. Thus follicle-stimulating hormone (FSH) acts on the germinal epithelium to promote full spermatogenesis, while interstitial-cell-stimulating hormone (ICSH) — hereafter called luteinizing hormone (LH) because of common usage — induces the interstitial cells to secrete androgens and estrogens.

There is some evidence that FSH is synergistic to the action of LH in Leydig cell steroidogenesis. The same may be true for spermatogenesis, although in this instance FSH would play the primary role and LH a secondary one. Indeed, it may be that LH acts as an indirect synergist through its stimulation of testosterone.

With respect to the pituitary testicular interrelationship, the control of LH secretion appears reasonably straightforward. When testosterone production is decreased by a primary testicular disease, LH secretion increases with an attendant rise in serum and urine LH titres. On the other hand, if testosterone secretion is pathologically increased, LH secretion is inhibited and LH titres are lowered.

The control mechanism for FSH secretion remains an enigma, and it is beyond the scope of this report to review the entire literature. However, certain points require emphasis. When the seminiferous tubules are severely damaged, FSH titres usually increase, even when plasma testosterone levels remain normal. Decreased sperm production has not been found to correlate directly with increased serum or urinary FSH titres.

The problem of elucidating the control mechanism for LH and FSH secretion has been further complicated by the isolation of a gonadotrophin-releasing hormone from the hypothalamus. This decapeptide has been shown to increase both LH *and* FSH secretion in the human male.

9.1 Fertility promoting agents

Gonadotrophin therapy has been carried out in the infertile male, using HMG, HPG, HCG, and HLH singly or in various combinations for the direct stimulation of testicular function.

The balance of evidence suggests that HCG or LH stimulates the mitochondrial activity of the Leydig cells, leading to a secretion of testosterone and other steroids.

The metabolic effects of FSH are less clear. Present information suggests that the principal effect of FSH is to activate nuclear protein

synthesis within the seminiferous tubular epithelium. It may be that the Sertoli cells are the primary target site for FSH action. However, the available data are insufficient to draw specific conclusions.

In order to determine the spacing of the injections to be used in therapy it is necessary to take into account the relative half-lives of HCG, HLH, and HFSH. With regard to HMG, there are no available data on the half-lives of its LH and FSH components. However, the HLH and HFSH data should afford approximate frames of reference. Each hormone exhibits a two-component plasma disappearance curve, and the circulating first and second half-life times are: for HCG 11 hours and 23 hours; for HLH 21 min and 3 hours 55 min; and for HFSH 3-4 hours and 70 hours.

Clomifene administration to males with an intact hypothalamo-pituitary system produces an increase in serum and urinary LH and FSH titres, but the response in men is different from that observed in women, there being, in particular, no abrupt LH surge.

It has been demonstrated that when clomifene is given to men, plasma testosterone increases. Since the metabolic clearance rate of testosterone remains unchanged, the increase must be due to greater production. Clomifene has been administered to men to test pituitary function and for the experimental treatment of idiopathic oligospermia.

Testosterone either alone or in combination with norethandrolone has been given to oligospermic males for the initial suppression of spermatogenesis. This treatment procedure, in the doses employed, inhibits FSH and LH secretion, although a direct suppressive action on the testis cannot be excluded. After the cessation of treatment an increase in spermatogenesis is sometimes noted (the so-called "rebound" phenomenon).

Some investigators have studied the effects of mesterolone — a synthetic testosterone analogue — in oligospermic males. It has been suggested that mesterolone does not suppress gonadotrophin secretion. In androgen-deficient men, the administration of mesterolone increases seminal fluid fructose levels. This finding suggests that mesterolone acts directly on the seminal vesicle receptors or perhaps alters the function of the Leydig cells in some direct fashion.

9.2 Patient selection

Two major groups of hypogonadal males have been studied to evaluate the therapeutic effects of gonadotrophin, clomifene, and testosterone and its analogues.

Group I: This group consists of:

- (a) hypophysectomized males
- (b) partial or complete hypopituitarism
- (c) hypogonadotrophic eunuchoid males.

To date the treatment of choice for this group of patients is gonadotrophin therapy, provided the goal is the restoration of fertility.

Group II: This group comprises idiopathic oligospermic males, including those with:

- (a) normal serum and urinary FSH levels
- (b) elevated serum and urinary FSH levels.

The Scientific Group felt that for this category of patients no specific gonad stimulating therapy can be recommended for the time being.

In general, progress in this field has been hampered by the inadequate definition of patients, the use of hormone assays of insufficient specificity (such as total gonadotrophin assays and urinary 17-ketosteroid determinations), and lack of appropriate standards for specific gonadotrophin assays.

Some of these deficiencies are being corrected, particularly by means of improved assay methods, the increasing availability of proper gonadotrophin standards, and adequate supplies of well-defined gonadotrophic preparations.

Males who have undergone hypophysectomy represent a varied pattern with respect to residual gonadotrophin secretion. Other portions of their central nervous systems may also be damaged. The same situation holds true for patients with partial or complete hypopituitarism. Careful attention must therefore be given to the basal hormonal status of these patients as well as to their overall medical status.

Hypogonadotrophic eunuchoidism is an autosomal dominant disorder with variable penetrance. Anosmia or hyposmia is frequently an associated finding. The classic form of the disease is that in which both LH and FSH secretion are deficient. The next most common form is the so-called "fertile" eunuch, who secretes varying amounts of biologically effective FSH but is deficient in LH. Accordingly, when testicular biopsy specimens are inspected from these patients, evidence of spermatogenesis may be found.

Since the hypothalmo-pituitary system is pathologically altered in hypogonadotrophic eunuchoidism, clomifene administration is ineffective. Preliminary data suggest that synthetic decapeptide (section 8) may induce LH and FSH secretion in these patients.

In conditions such as Klinefelter's syndrome, post-mumps orchitis, varicocele, diseases of the epididymis or vas deferens, and Reifenstein's syndrome,¹ the precise mechanisms that lead to gonadal damage and impaired spermatogenesis are incompletely understood.

¹ Bowen, P., Lee, C.S., Migeon, C.J., Kaplan, N.M., Whalley, P.J., McKusick, V.A. & Reifenstein, E.C. (1965) Hereditary male pseudohermaphroditism with hypogonadism, hypospadias, and gynecomastia (Reifenstein's syndrome), *Ann. intern. Med.*, **62**, 252.

9.3 Treatment results

9.3.1 *Gonadotrophins and clomifene*

Group I. Present information indicates that most of the patients in Group I (*a*) and (*b*) respond to exogenous gonadotrophin therapy by showing normal circulating testosterone levels and complete spermatogenesis. The volume, composition, and sperm concentration of the seminal fluid are also normalized. On the other hand, some patients in this category have suffered from gonadotrophin deficiency for so long without treatment that they exhibit complete hyalinization of the interstitium and the peritubular membranes. Therefore the Scientific Group recommended that patients in categories I (*a*) and (*b*) undergo testicular biopsy to assess their responsiveness to proposed gonadotrophin therapy, since complete hyalinization precludes a positive therapeutic response.

It has been established that the administration of HPG + HCG can stimulate testicular function in patients belonging to Group I (*c*). In some patients HMG needs to be added to the HCG for full spermatogenesis. The successful induction of normal spermatogenesis from an immature state, with normal Leydig cell function and consequent normal androgen production, has been accomplished with documented pregnancies.

The data on HLH treatment are still provisional. However, it has been established that HLH in daily doses of 400 IU can stimulate the Leydig cells to secrete normal amounts of testosterone. The daily dose of FSH in HMG or HPG required for satisfactory results appears to be between 75 and 225 IU. If administered 3 times weekly, the dose should be 150-225 IU per injection.

The amount of LH that has to be administered together with FSH (in the form of HMG or HPG) has not been established. For the time being, to achieve adequate stimulation of the Leydig cells, doses of 2 000-5 000 IU of HCG are administered 2 or 3 times a week, usually concomitantly with HMG or HPG preparations.

Group II. The data available indicate that some patients in Group II respond to therapy with gonadotrophins and/or clomifene, but it is difficult at this stage to identify them because of the various treatment protocols employed in previous studies and because of deficiencies in the determination of hormonal and genetic status and in the pretreatment evaluation of seminal fluid. In view of the wide variations in sperm counts that occur in both normal and oligospermic males, the practice of obtaining single or even three pretreatment seminal fluid specimens appears to be inadequate. Therefore new techniques will have to be employed to assess the treatment of these patients.

The Scientific Group concluded that the efficacy of gonadotrophin or clomifene treatment in Group II males remains to be established.

9.3.2 *Testosterone and mesterolone*

In one series of 163 courses of treatment carried out in 157 males in Group II (a) and (b), therapy based on the "rebound" phenomenon using testosterone (with or without norethandrolone) resulted in a 41% incidence of conception. In other tests the incidence of "good results" (either increased sperm counts and/or pregnancies) ranged from 3.9% to 18.5%. The Scientific Group concluded that, since the rationale of this form of therapy requires clarification and since the results of treatment have been so variable, further studies carried out in a more systematic fashion are required before the efficacy of the testosterone "rebound" treatment can be defined.

The Scientific Group considered that the data on mesterolone therapy are insufficient to be evaluated.

10. RECOMMENDATIONS FOR RESEARCH

A. Research of high priority

(1) Determination of the optimum conditions for the induction of ovulation by releasing hormones, to be carried out on a collaborative basis.

(2) Determination of fetal wastage in normal and induced gestation, using precise HCG assay, to be carried out on a collaborative basis.

(3) Collaborative development of uniformly acceptable protocols for the clinical evaluation of various gonad-stimulating agents in both the female and the male.

(4) Collaborative development of mathematical models for improving the detection of changes in the concentration of spermatozoa in seminal fluid.

(5) Determination of the prevalence of infertile individuals who would benefit from treatment with gonad-stimulating agents.

(6) Assessment of children born after induced ovulation, by means of follow-up studies.

(7) Improvement of methods for the assay of gonadotrophin-releasing hormones.

(8) Use of various formulations of releasing hormones and determination of the best routes of administration for diagnostic and therapeutic purposes, including a search for possible metabolic and adverse effects.

(9) Establishment of suitable international reference preparations and standard antisera for the radio-immunoassay of FSH and LH in plasma.

(10) Determination of the separate effects of FSH and LH and their subunits, for which purpose FSH preparations containing little or no LH and LH preparations containing little or no FSH would be needed, as well as suitable preparations of subunits.

B. Other recommended studies

Male and female

(1) Development of functional diagnostic tests for the classification of infertile patients.

(2) Elucidation of the effect of gonadotrophins and their subunits on ovarian and testicular steroidogenesis.

(3) Investigation of antibodies to factors other than gonadotrophins by means of immunological studies in gonadotrophin-treated patients.

(4) Determination of the effect of drugs on ovarian and testicular response to gonadotrophins, including various neuropharmacological agents such as levodopa. (These drugs should be administered either prior to, or together with, gonadotrophins and should include various estrogens, progesterone, progestogens, and clomifene.)

(5) Investigation of the mechanism of action of FSH, LH, and HCG and of their subunits at the subcellular and cellular levels.

(6) Determination, by clinical studies, of the mechanism of action of steroid hormones and pituitary hormones in higher brain centres and of the relationship between releasing hormones and pituitary function.

(7) Investigation of steroid metabolism in the central nervous system.

(8) Investigation of the mechanism of action of releasing hormones at the cellular and subcellular levels.

(9) Development of long-acting formulations of FSH, LH, and HCG.

(10) Classification, by means of model experiments, of gonad-stimulating agents inducing an increase of FSH and LH. Such studies could be suitably carried out in normal males.

(11) Development of factors that release mainly FSH.

Female only

(1) Recovery and study of oöcytes after the induction of ovulation.

(2) Study of oöcyte maturation, especially in relation to the LH surge.

(3) Comparative study of hormonal patterns in plasma and urine in induced ovulation and in spontaneous ovulation (e.g., by determining estrogen metabolism at the time of ovulation).

(4) Development of functional tests to differentiate different types of luteinization, the ovarian histology being correlated with hormonal changes.

(5) Study of hormonal changes in natural early pregnancies and in induced pregnancies.

(6) Investigation of possible autoimmune causes of secondary ovarian failure.

(7) Study of age-related failure to conceive after ovarian stimulation.

(8) Study of conditions in which various steroids (particularly intravenously administered estrogens) induce a release of gonadotrophins.

Male only

(1) Development of improved methods of assessing the fertilizing ability of sperm.

(2) Investigation of the factors that may be involved in the pathogenesis of idiopathic oligospermia.

Annex

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