IMMUNOCHEMICAL ASSAY OF FOLLICLE-STIMULATING AND LUTEINIZING HORMONE IN THE UNCONCENTRATED URINE OF SUB- OR INFERTILE MALES

By Joachim Mauss1) and Gereon Börsch2)

ABSTRACT

The excretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) was assayed in unconcentrated 2 × 24 h urine by means of a haemagglutination inhibition reaction FSH-Nosticon and Luteonosticon. The assay was performed in 10 fertile males and 121 sub- or infertile male patients. Normal values were 4.2 (range x ± 2 s: 1.8–9.9) IU/24 h for FSH and 12.9 (5.9–28.2) IU/24 h for LH (by immunoassay, standard: 2nd IRP-HMG). A significant inverse correlation could be established between the log sperm concentration and log FSH excretion (r = −0.68; n = 126; P < 0.001). No correlation could be found between either motility or morphology and log FSH or log LH excretion. In 87 patients with normal testicular size regardless of sperm counts, LH excretion was above the normal range only 6 times. However, in uni- and bilateral testicular atrophy and furthermore in patients with varicocele, the mean LH excretion was significantly elevated in comparison to normal levels.

Some years ago, a simple immunochemical test for the determination of LH in unconcentrated urine became commercially available (Schuurs & van Wijngaarden 1970). The method proved to be very suitable for use in daily labora-

1) Request for reprints must be mailed to Priv. Doz. Dr. J. Mauss, Evang. Krankenhaus, 4 Düsseldorf, Postfach 2940, FRG.
2) Part of a doctoral thesis.
tory routine. Several investigators presented reports on LH determinations in male sub- and infertility (Muller et al. 1970; Mauss & Böke 1972; Mauss 1972; Krause & Monadjemi 1972). The main finding was the lack of correlation between sperm count and LH excretion. Recently a similar test for the assay of FSH in unconcentrated urine (Schuurs & van Wijngaarden 1972) became available for clinical evaluation. Since data in the literature concerning FSH levels in oligozoospermia are inconsistent, we decided to conduct an investigation on a larger number of sub- and infertile males, special attention being paid to the possible existence of a correlation between sperm count and FSH excretion. This work, supplemented by determinations made with a new LH test with increased sensitivity forms the subject of the following report1).

MATERIALS AND METHODS

Subjects

Normal values were established in 10 healthy males between 22 and 35 years of age, with normal spermiograms as judged by the criteria mentioned below and testes larger than 12 ml in volume as compared with an orchidometer (Schonfeld 1943). The subjects collected 2 to 4 24 h urine samples during normal daily routine, making up a total of 37 samples.

The group of patients consisted of 121 males aged 20 to 50, who were examined in our andrology department between August and November 1972 for barren marriage. For admittance to this study one or more of the following criteria had to be met: sperm counts below 40 million spermatozoa per ml, motility below 40%, morphology below 50% normal forms, or seminal fructose below 100 mg/100 ml. Careful clinical examination and determination of testicular volume using an orchidometer were performed in each case. Diagnosis of Klinefelter's syndrome was established or excluded by buccal smear analysis. All patients collected 2 24 h urine samples on consecutive days, usually over a work-free week-end, reducing fluid intake during days of sampling. Appropriate portions of all urines were kept frozen at −20°C until the day of assay.

Spermiograms

Diagnosis was based on at least two spermiograms, ejaculations being obtained by means of masturbation after a period of sexual continence of 4–6 days. The following characteristics were established: volume, pH and viscosity of ejaculate, sperm count per ml using a haemocytometer, motility by recording the percentage of motile forms within 60 min after ejaculation, and percentage of normal morphology. Seminal fructose was estimated by the method of Doepfmer (1960).

Assay of FSH and LH

Urinces were assayed using haemagglutination inhibition reactions as described by Schuurs & van Wijngaarden (1972). This is based on the method of Wide et al. (1961) for measuring urinary LH with the modification, that the antigen-antibody reaction is

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1) FSH-Nosticon and Luteonosticon. Organon Teknika Int. B. V., Oss/The Netherlands.
except zoospermia different to Sperm Student's cated of assay, Normal groups various assays. experienced evaluation of 24 incubation of the endpoint. of sediment. an addition enabling allowed to occur in a large volume (Schuurs 1970), thus augmenting the sensitivity and enabling the assay to be made in unconcentrated urine. Urines are prepared by centrifugation (3,000–4,000 r. p. m. for 10 min), heating (80°C for 10 min), treatment with an adsorbent and filtration. Dilution series with a dilution factor 2 are set up for the assay of FSH and LH, respectively, starting with a volume of 6 ml urine, and the addition of antisera to FSH and HCG, respectively. After a pre-incubation period of 16–20 h, erythrocytes coated with FSH and HCG, respectively, are added. Erythrocytes are agglutinated by antisera to FSH and HCG, while free urinary FSH and LH in sufficient concentrations inhibit agglutination by neutralizing the antisera. After an incubation period of 90 min, erythrocytes are removed from the test fluid by means of centrifugation (700 × g for 10 min), washed once and transferred to sufficiently small round-bottomed sedimentation tubes, where agglutination inhibition leads to a distinct ring formation within two hours, while agglutination results in a diffuse brown sediment.

The highest dilution giving a complete agglutination inhibition is taken as the endpoint. The tests are adjusted to give an agglutination inhibition in the presence of 2 IU FSH/l and 8 IU LH/l or more, respectively, in the test fluid. The choice of the assay endpoint is to some extent subjective (Arquila & Stavitsky 1956; Schuurs 1970) and a matter of definition. Therefore, to ensure a standardized and constant evaluation of sedimentation patterns, all assays were performed and read by the same experienced technician. Though there may be differences in absolute values between various laboratories, the method thus standardized permits a comparative study of groups of subjects, as well as of the effects of various stimuli upon one individual. Each 24 h sample was assayed separately and values are reported as the mean of both assays. To fulfill normality assumptions, all values were transformed by taking the log of the original values before calculations.

RESULTS

Normal values

Values computed as the geometrical mean of assays on the 37 urine samples of 10 normal males were 4.2 (range x ± 2 s: 1.8–9.9) IU/24 h (by immunoassay, standard: 2nd IRP-HMG) for FSH and 12.9 (5.9–28.8) IU/24 h (by immunoassay, standard: 2nd IRP-HMG) for LH. This normal range x ± 2 s is indicated in all figures by hatched horizontal lines.

Sub- or infertile males

Sperm count

FSH. – The mean FSH excretion in groups of patients classified according to their sperm concentration increased progressively (Fig. 1). As judged by Student’s t-test, the mean excretion values of patients with azoospermia, oligozoospermia of 1–4 and 5–9 million spermatozoa per ml were significantly different from each other (P < 0.001) as well as from all other groups (P < 0.01), except the difference between groups of 1–4 and 5–9 million per ml (P > 0.4).
FSH excretion in normal males, 117 sub- or infertile patients arranged according to their sperm concentration, and 3 patients with Klinefelter's syndrome. Normal range is indicated by hatched horizontal lines.

While FSH excretion in azoospermic males was uniformly elevated, all oligozoospermic groups showed a wide range from normal to elevated FSH levels. The mean FSH excretion of 3 patients with Klinefelter's syndrome could not be distinguished from the azoospermic group ($P > 0.7$).

There was a significant inverse correlation between log sperm concentration and log FSH excretion in all patients and normal subjects excluding patients with Klinefelter's syndrome (Fig. 2). For the purpose of calculating logarithms, azoospermic patients were assigned the arbitrary value log 0.5 million spermatozoa per ml equivalent to 5.7. The correlation between log sperm concentration and log FSH excretion remained significant, when patients with azoospermia were excluded ($r = -0.59; n = 114; P < 0.001$), but ceased to be significant, when the group with sperm counts of 10 million per ml or more was analysed separately ($r = -0.24; n = 64; P > 0.05$).

LH. – Though the mean LH excretion of groups of patients with normal testicular size, classified according to their sperm concentration, showed some tendency towards increased levels when the mean sperm concentration decreased, only 6 values above the normal range were observed (Fig. 3).
Fig. 2.
Correlation between log sperm concentration and log FSH excretion in 126 males (regression equation: log FSH = 2.75 - 0.25 × log sperm concentration). Normal range is indicated by hatched horizontal lines.

Fig. 3.
LH excretion in normal males, 87 sub- or infertile patients with normal testicular size arranged according to their sperm concentration, and 3 patients with Klinefelter's syndrome. Normal range is indicated by hatched horizontal lines.
Motility and morphology

*FSH.* – The influence of motility and morphology on FSH excretion was analysed in the sperm count range in which a correlation with log FSH excretion ceased to be significant. Thus, in all males with sperm counts of 10 million spermatozoa per ml or more, there was no significant correlation between motility and log FSH excretion ($r = -0.08; n = 64; P > 0.1$) and morphology and log FSH excretion ($r = -0.15; n = 64; P > 0.1$).

*LH.* – In all males with a normal testicular size, there was neither a significant correlation between motility and log LH excretion ($r = -0.05; n = 84; P > 0.1$) nor between the morphology and log LH excretion ($r = -0.18; n = 81; P > 0.1$).

Genital characteristics

Fig. 4 shows the urinary LH excretion of groups of patients classified according to genital characteristics. There was no significant difference in LH excretion between normal males and patients with normal testicular size ($P > 0.2$). In comparison to these groups, the mean LH excretion was slightly elevated in patients with normal testicular size and varicocele ($P < 0.02$). The

Fig. 4.

LH excretion in normal males, 117 sub- or infertile patients arranged according to their genital characteristics, and 3 patients with Klinefelter's syndrome. Normal range is indicated by hatched horizontal lines.

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most marked increase was found in patients with unilateral and bilateral testicular atrophy and Klinefelter's syndrome. Differences between these three groups were not significant ($P > 0.1$); but mean LH excretion was significantly elevated as compared with all three groups mentioned initially ($P < 0.5$). With only one exception, all males with LH levels above the normal range also had an increased FSH excretion above the normal range.

**Hypogonadotrophic hypogonadism**

Only one patient out of 121 showed clinical signs of hypogonadotrophic hypogonadism. He was azoospermic with bilateral testicular atrophy; testicular biopsy revealed pre-puberal testes. The basal FSH and LH excretion of the 32 year old subject was determined on 4 occasions and averaged 2.4 IU/24 h and 8.7 IU/24 h, respectively. FSH and LH excretion was again determined on day 10–13 during the administration of 200 mg clomiphene citrate/24 h$^1\)\. The FSH levels averaged 3.4 IU/24 h, which is not significantly different from pre-treatment levels ($P > 0.05$), while the mean LH excretion decreased significantly (6.3 IU/24 h; $P < 0.02$).

**DISCUSSION**

Our normal values compare well with other data in the literature, obtained either by specific bioassays (Christiansen 1972a) or by radioimmunoassay (Franchimont 1970). Deviation from a previously reported normal value for LH excretion from this laboratory, obtained with a different LH-test (Mauss & Böke 1972), is attributed to the increased sensitivity (8 IU LH/l vs. 25 IU LH/l), involving different dilution steps (8, 16, 32 ... IU LH/1 vs. 25, 50, 100 ... IU LH/l), of the modified LH-test used in this investigation.

Our finding of a significant inverse correlation between log sperm concentration and log FSH excretion in 126 males clearly points to a close relationship between FSH levels and spermatogenesis, as was previously observed by Kaivola & Johansson (1969), Hilfrich et al. (1970), Franchimont (1970, 1971), Christiansen (1972b), Wieland et al. (1972), van Thiel et al. (1972) and Kjessler & Wide (1973). Our data support especially those of Johnsen (1970), who found a negative correlation between log sperm count and log total urinary gonadotrophin in 158 males ($r = -0.54; P < 0.001$) as well as those of Rosen & Weintraub (1971), who reported an even greater negative correlation between sperm count and serum FSH levels in 17 patients with idiopathic oligo-azoospermia ($r = -0.65; P < 0.01$). Of further interest is the fact that a correlation between

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$^1\) Dyneric®, Merrell Pharma.
log sperm concentration and log FSH excretion ceased to be significant for the sperm count range of 10 million spermatozoa per ml or more. This agrees favourably with the data of MacLeod & Gold (1953) that fertility is impaired when the sperm count drops to below 20 million per ml. Thus sperm counts of 10–20 million per ml seem to represent the stage of damage to the germinal epithelium, at which fertility as well as testicular feedback inhibition of pituitary FSH release by an unknown mediator become markedly affected.

While there is general consensus of opinion regarding elevated FSH levels in azoospermia not due to occluded deferential ducts or to hypothalamo-pituitary disturbances, several investigators could not confirm the findings of elevated levels in oligozoospermia (Franchimont et al. 1972; de Kretser et al. 1972; Leonard et al. 1972).

Both latter groups determined FSH concentrations in single plasma samples by radioimmunoassay. However, recent findings of a pulsatile secretion pattern of FSH and LH from the pituitary in the male (Nankin & Troen 1971, 1972; Boyar et al. 1972a,b, 1973; Root et al. 1972; Murray & Corker 1973; Naftolin et al. 1973) have clearly demonstrated some limitations of the value of these single determinations. Nevertheless, we agree with these investigators in so far as all our oligozoospermic groups showed a wide range from normal to elevated FSH excretion levels, as was very recently found again by Kjessler & Wide (1973), using bioassay in 24 h urines of 536 males. Observations on 28 testicular biopsies from patients of this study indicate that markedly elevated FSH levels are only found in those cases having bilateral germinal maturation arrest at or before the spermatocyte stage or a Sertoli cell syndrome (Börsch et al., in press).

Studies of the pituitary gonadotrophin reserve of those males with normal FSH levels despite oligozoospermia below 10 million per ml revealed an adequate response to clomiphene and LH-releasing hormone stimulation (Mauss et al. 1973). Thus, a partial block to the egress of sperm (Rowley & Heller 1972), leaving spermatogenesis undisturbed, is the most likely explanation for this condition.

The observation of predominantly normal LH excretion levels in sub- or infertile patients with normal testicular size corresponds well with the findings of Rosen & Weintraub (1971), Franchimont et al. (1972) and Mauss & Böke (1972) that LH levels are not related to spermatogenesis. Nevertheless, a certain tendency towards increased levels was apparent in patients with low sperm counts. Correlation between log sperm concentration and log LH excretion in males with normal testicular size was significantly different from zero (r = −0.41; n = 96; P < 0.001) and of approximately the same degree (r = −0.46) as that observed by Rosen & Weintraub (1971) between sperm concentration and serum LH levels, but only 6 values above the normal range were found.

However, LH excretion was frequently elevated in patients with testicular
atrophy a finding previously reported by Mauss & Böke (1972), indicating a severe impairment of endocrine testicular function in this condition. The frequency of elevated LH levels in patient with oligo- or azoospermia found by Wide & Kjessler (1969), de Kretser et al. (1972) and Kjessler & Wide (1973) may be due to a considerable number of males with testicular atrophy among their patients, since no reference to testicular size is given by these investigators.

The slightly but significantly elevated LH levels in patients with a varicocele indicate an impairment of Leydig cell function. Raboch & Stárka (1971) demonstrated low plasma testosterone levels in a similar group of patients.

As far as we know, the lack of correlation between FSH and LH levels and spermatozoa motility and morphology has not previously been mentioned in the literature.

No patients showed gonadotrophin excretion below the normal range. The values of our patient classified as hypogonadotrophic were in the low normal range. Low normal values, in cases of hypogonadotrophic hypogonadism, obtained by immunoassay, have been demonstrated among others by Albert et al. (1968), Ross (1970), Franchimont (1971) and Santen et al. (1971). The diagnosis in our patient was clearly established by the findings of pre-puberal testicular biopsy specimen and a negative response to clomiphene stimulation.

With the tests for simultaneous determination of FSH and LH in unconcentrated urine as applied here, the pituitary-testicular axis can be assessed quantitatively in any andrological laboratory. In addition to assessment of basal levels, the tests proved to be sensitive enough to allow evaluation of clomiphene and LH-releasing hormone stimulation of the pituitary (Mauss et al. 1973). For the large majority of sub- or infertile males, the hormonal state thus established saves fruitless gonadotrophin therapy.

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