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Plasma and Urinary Follicle-Stimulating Hormone Levels in Oligospermia

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ABSTRACT. Plasma and urinary FSH levels were measured in adult men with oligospermia or azoospermia. Patients with elevated plasma LH levels were excluded. In 60 oligospermic subjects the mean plasma FSH concentration (415 ± 175 SD ng LER 907/ml) was not significantly higher than that of 42 control subjects with normal spermatogenesis (355 ± 76). Only in the azoospermic patients was the plasma FSH uniformally elevated (865 ± 380). No direct reciprocal relationship between sperm count and plasma FSH was evident in the oligospermic group ($r = -0.18$, $p > 0.10$). In 12 additional oligospermic men the quantitative histologic characteristics of testicular biopsy specimens were compared with urinary FSH levels. There was no correlation between the germinal cell populations and FSH titers. These data do not support the thesis of a direct reciprocal relationship between spermatogenic activity and FSH secretion but suggest the presence of an "independent testicular station," yet undefined which controls FSH. (*J Clin Endocr* 34: 209, 1972)

IT IS APPARENT that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion are regulated independently in men. Support for this is derived from a number of observations. Castration is followed by an increase in the plasma concentration of both FSH and LH (1,2) but only LH levels are suppressed by androgens administered in physiologic amounts (3,4). Also, selective damage to the seminiferous tubules by radiation results in an increase in FSH without any significant effect on testosterone or LH levels (5). These and other studies would indicate the relationship between LH and testosterone is analagous to that between ACTH and cortisol. In contrast, our understanding of the interrelationship of FSH secretion and testicular function is fragmentary.

Although the mechanism by which the testes exert a feedback inhibitory effect on follicle-stimulating hormone secretion remains unknown, it has been postulated that the germinal cells are the source of a humoral substance which inhibits FSH secretion (6, 7). The major support for this concept is based on investigations which have reported a reciprocal relationship between spermatogenesis and urinary gonadotropin titers (8,9). This concept has been challenged by others who were unable to find an inverse relationship between spermatogenesis and urinary gonadotropin excretion (10–12). Subsequent to these earlier studies specific methods for measuring FSH have been developed (13,14). The present study was initiated to determine by utilizing these methods whether FSH secretion is reciprocally related to spermatogenesis.

Materials and Methods

Subjects. Forty-two men in good health with normal spermatogenesis (mean sperm count >60 million/ml), ranging in age from 22 to 51 years, were selected to serve as the normal control population for measurement of plasma FSH. Clinically these men were androgenically normal and had normal plasma levels of LH.

Seventy-six patients, age 21 to 45 years, with either oligospermia (sperm count <40 million/ml) or azoospermia were studied. Clinically they were androgenically normal and had normal plasma LH levels. Oligospermic or azoospermic patients with elevated plasma LH values were excluded. In sixty-four, measurement of plasma FSH was made and correlated with the

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FIG. 1. Representative seminiferous tubule selected for germinal cell quantitations. (×640)

seminal fluid sperm concentration. The remaining twelve oligospermic subjects had testicular biopsies performed and the quantitative histologic characteristics of the seminiferous tubules were correlated with urinary FSH levels. Only patients in whom complete information was available regarding seminal fluid analyses, urinary FSH levels and testicular biopsies were included in this latter group.

Plasma FSH and LH. Plasma FSH was measured by the radioimmunoassay technique of Midgley (14). All reagents for this immunoassay were generously supplied by the National Pituitary Agency. Separation of antibody bound from free hormone was accomplished by the addition of sheep antirabbit gamma globulin serum in sufficient quantity to achieve maximal precipitation. Human Pituitary Reference Preparation LER 907 served as the standard. In our laboratory the lower limit of sensitivity of the FSH assay is 78 ng/ml. The intra-assay and interassay coefficients of variation for a mid-slope plasma pool were 5% and 13%, respectively. Plasma LH was measured by the double antibody radioimmunoassay method of Morgan *et al.* (15) as adapted by Midgley for LH (16). The Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP) served as the LH standard; the precision and sensitivity of the LH assay in this laboratory have previously been reported (17).

Urinary FSH. Urinary FSH titers were determined by the Steelman-Pohley method (13) after kaolin-acetone precipitation (18). Titers are expressed as IU/24 hr; the 2nd IRP was used as the reference preparation. Relative potency estimates were determined by the parallel line assay computer program of Thorslund and Paulsen (19). The normal adult male range by this method in our laboratory is 3.8–25.0 IU/24 hr (17), although occasionally urinary FSH can only be detected intermittently in a 12-hour aliquot.

Seminal fluid analysis. The seminal fluid sperm concentrations were determined by either the

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standard hemocytometer (20) or Coulter-counter methods (21) in a minimum of three specimens per study subject. The mean number of semen analysis performed in the azoospermic and oligospermic group was nine. A subject was considered to be azoospermic only when sperm were absent in all seminal fluid specimens analyzed. A smear of undiluted seminal fluid was examined in each analysis and if any sperm were seen the patient was not considered to be azoospermic. A minimum of nine semen analyses were performed in each azoospermic subject. In addition, analysis included determination of sample volume and motility of spermatozoa.

Quantitative analysis of the germinal epithelium. Testicular biopsy was performed under local anesthesia and the tissue immediately placed into freshly prepared Cleland's fixative. Germinal cell quantitation was performed on the stained sections in the following manner: A low power photomicrograph of an entire testicular biopsy section was taken and those tubules which had been cut perpendicular to their long axis were identified and numbered consecutively. Tubules cut at oblique angles were disregarded. Fig. 1 shows the type of tubule so selected for examination. The numbered tubules were then examined under the light microscope and the individual germinal cells were identified using the cytologic criteria of Clermont (22). The germinal cells were counted in ten tubules per biopsy specimen and the resultant counts expressed as the mean cell count per tubule. The cell types scored separately in this manner were: A dark, A pale, and B spermatogonia; resting, leptotene-zygotene, and pachytene primary spermatocytes; spermatids.

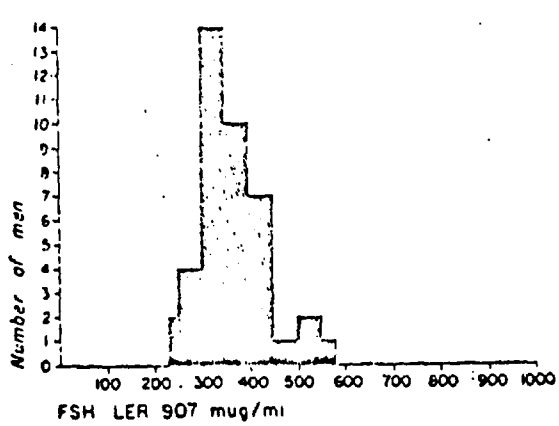

FIG. 2. Plasma FSH in 42 normal adult males.

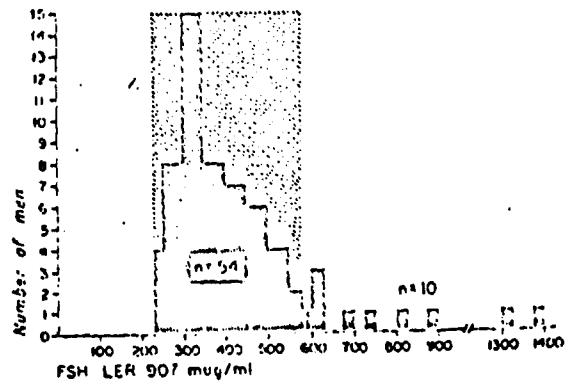

FIG. 3. Plasma FSH in 60 patients with oligospermia and 4 patients with azoospermia. Cross-hatched area indicates the range of plasma FSH in the normal control population.

Results

The range of plasma FSH in the adult male control population with normal spermatogenesis was 250–580 ng LER 907/ml with a mean value of 355 ± 76 (SD) ng/ml (Fig. 2). All had plasma LH concentrations within the normal range of our laboratory, 4–19 mIU/ml 2nd IRP.

Fig. 3 presents the plasma FSH levels in sixty-four of the patients with oligospermia or azoospermia. Fifty-four of these subjects had FSH determinations within the range observed in the control population. Of the remaining ten patients four had FSH levels slightly above the control range and in six the plasma FSH was clearly elevated. The mean level for the entire group was 444 ± 218 (SD) ng/ml, for those with only oligospermia 415 ± 175 (SD) ng/ml and 865 ± 380 (SD) ng/ml in those with azoospermia. No direct reciprocal relationship between seminal fluid sperm concentration and plasma FSH levels was evident in the oligospermic group ($r = -0.18$, $p > 0.10$) (Fig. 4). The plasma FSH concentration was elevated in all azoospermic subjects studied (Fig. 4). The mean level of plasma LH for the oligo-azoospermic group was 11.0 ± 4.4 (SD) mIU/ml.

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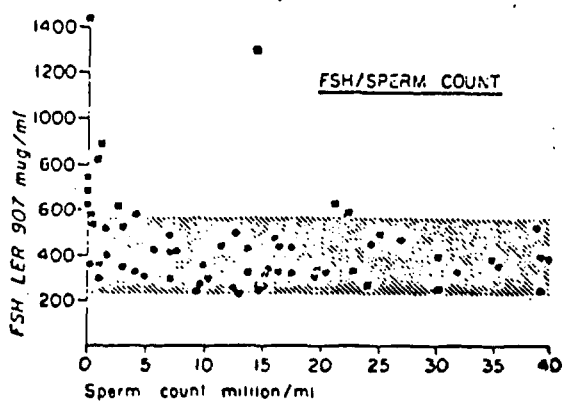

FIG. 4. Comparison of plasma FSH and mean sperm count in oligo-azoospermic patients. Cross-hatched area represents the range of plasma FSH in the normal control population.

Germinal cell quantitation and urinary FSH titers. The quantitative histologic data of the testicular biopsies in twelve oligospermic patients are shown in Table 1. These men were divided into two groups on the basis of their urinary FSH titers. The age of the patients and severity of oligospermia in the two groups were comparable. There was no statistically significant difference between these two groups in any of the specific germinal cell counts and, therefore, no direct reciprocal relationship between the germinal cell populations and urinary FSH titers was present.

Discussion

Our data are contrary to the expected results and raise important questions about the testicular feedback mechanism for regulating FSH secretion in the male. The concept that the germinal epithelium of the testes is the source of a humoral substance which inhibits pituitary gonadotropin secretion can be traced to the studies of Mottram and Cramer (6). They observed the appearance of castration cells in the rat pituitary following radiation-induced degeneration of the germinal epithelium. Since morphologically the Leydig cells did not appear damaged by the dose of radiation administered in their studies and the secondary sex glands did not atrophy, they postulated that the germinal epithelium normally produces a

TABLE 1. Histologic data of testicular biopsies in 12 oligospermic subjects

Subject	Age (yr)	FSH (IU/24 hr)	Sperm count (million/ml)	Mean germinal cell count/tubule†						
				Ad	Ap	B	R	LZ	P	Sp
I. Normal FSH										
T.J.	25	18.2	2.5	25.4	11.8	7.1	0.4	10.0	51.0	66.0
W.C.	29	16.5	8.0	14.8	4.8	2.6	0	6.0	14.3	20.0
J.V.	43	8.0	1.6	15.8	4.7	4.3	0.9	13.2	42.1	70.1
C.A.	21	9.0	24.0	18.8	2.4	3.4	1.2	5.6	32.0	76.2
B.C.	28	3.0	37.0	16.2	3.8	3.1	0.8	4.5	26.0	64.3
H.M.	36	12.0	0.3	14.5	4.4	2.7	1.3	12.9	20.4	0
C.M.	31	10.0	7.0	20.0	2.8	3.9	1.2	7.2	38.3	47.3
Mean		11.0	11.5	17.9	4.9	3.9	0.8	8.5	32.0	49.1
±SD		± 5.2	±13.8	± 3.9	± 3.2	±1.5	±0.5	± 3.6	±12.8	±28.7
II. Elevated FSH										
L.R.	27	31.0	7.8	9.6	3.5	4.1	0.6	7.2	21.8	26.1
C.S.	40	26.0	11.6	24.5	5.0	6.6	0	12.5	44.7	54.9
E.R.	33	36.0	13.2	13.6	4.3	3.3	0	6.8	26.7	33.8
R.S.	24	54.0	24.2	10.4	3.7	4.7	0	8.0	27.7	49.8
E.K.	26	26.0	8.3	13.7	2.0	2.8	1.1	4.9	27.3	7.8
Mean		34.6	13.0	14.4	3.7	4.3	0.3	7.9	29.6	34.5
±SD		±11.6	± 6.6	± 6.0	± 1.1	±1.5	±0.5	± 2.8	± 8.7	±18.9

† Abbreviations: spermatogonia = Ap (A pale), Ad (A dark), B; primary spermatocytes = resting (R), leptotene–zygotene (LZ), pachytene (P); spermatids (Sp).

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hormone which has an inhibitory effect on gonadotropin secretion.

The results of investigations directed toward correlating gonadotropin excretion and spermatogenic activity in human subjects have been conflicting. The majority of oligospermic subjects by Snijfen, Howard and Simmons (12) had normal urinary gonadotropin levels. Nelson (10) reported that urinary gonadotropin titers were "usually normal" in oligospermia; this statement was based on urinary gonadotropin titers in over 378 infertile males with varying degrees of oligospermia. Heller *et al.* (8) correlated gonadotropin excretion and testicular histology in 177 infertile males and concluded that as spermatogenic activity decrease gonadotropin titers increased. More recently Johnsen (9) also reported an inverse relationship between spermatogenic activity and gonadotropin excretion and has suggested that the cytoplasm which is split off from the maturing spermatid is the source of a substance which inhibits FSH secretion. There are a number of possible reasons for these divergent findings. All of the above studies utilized various bioassay techniques for assessing gonadotropin excretion which did not distinguish between FSH and LH. The precision of these methods was limited by the very nature of the bioassay end-points of increases in uterine and ovarian weight. Also, valid laboratory parameters for assessing Leydig cell function were not available to earlier investigators and subjects with varying degrees of Leydig cell failure may have been included in their studies.

The present studies employed specific methods for measurement of plasma and urinary FSH and only subjects with normal plasma LH levels were included. Oligospermic or azoospermic patients encountered during the course of this study in whom plasma LH levels were found to be elevated were purposely excluded from consideration. The validity of using plasma LH levels as an indirect assessment of Leydig cell function has been demonstrated by Paulsen *et al.* (17). They noted in patients with Klinefelter's syndrome, a condition in which a variable but almost universal failure of Leydig cell function occurs, that plasma testosterone concentrations were frequently normal; twelve of their twenty-five patients had normal plasma testosterone concentrations. In contrast, plasma LH levels were distinctly elevated in all cases but one. Their findings indicate that the plasma LH concentration may be a more reliable index of Leydig cell function in subjects in whom the hypothalamic-pituitary axis is intact than the measurement of plasma testosterone alone.

According to the studies of MacLeod and Gold (23) oligospermia is most appropriately defined as a sperm count of <20 million/ml. We have included in our oligospermic group patients with sperm counts of 20–40 million/ml in whom motility of the spermatozoa was also impaired. However, even when only those oligospermic subjects with sperm counts <20 million/ml were examined no correlation between FSH levels and spermatogenesis could be made ($r = -0.17$, $p > 0.10$).

The suggestion of Johnsen (9) that the testicular feedback mechanism for inhibiting FSH secretion is dependent on the very late stages of spermatogenesis when the residual body is split off from the spermatid does not appear likely in view of our data. We could find no correlation between any of the germinal cell types and urinary FSH levels. Of particular interest in this regard are the findings in subject H.M. (Table 1). This patient had severe oligospermia, a sperm count <1 million/ml on all seminal fluid examinations, and no spermatids were present in the ten tubules selected for germinal cell quantitation. Moreover, fewer than six spermatids were seen in a cross section of the entire testicular biopsy specimen and yet urinary FSH titers remained well within the normal range.

Rosen and Weintraub (24) recently reported an increase in mean serum FSH concentration in a group of patients with either oligospermia or azoospermia. They also noted that the majority of oligospermic sub-

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jects had serum FSH levels within the range observed in their control population of normal adult males. In contrast, FSH was elevated in all patients with azoospermia. These observations are in agreement with our findings. Although serum FSH concentration was found by them to be inversely correlated with sperm concentration when both the azoospermic and oligospermic subjects were analyzed together, there does not appear to be any significant correlation between sperm concentration and serum FSH when the oligospermic group is analyzed separately.

Our data indicate that the germinal cells do not play a role either directly or indirectly in the feedback regulation of FSH secretion but that this is a function of "an independent testicular station," as yet undefined. The integrity of this "independent testicular station," would not then be directly related to spermatogenic activity but might be disrupted by some of the same underlying factors or conditions causing oligospermia.

Acknowledgments

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