Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 1037–1041. Pergamon Press. Printed in Great Britain.

RELATIONSHIP BETWEEN GONADOTROPHINS, SPERMATOGENESIS AND SEMINAL PLASMA

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SUMMARY

The correlations between basal FSH and LH levels, FSH and LH response to LH RH injection, sperm count, stage of spermatogenesis and prolactin levels have been investigated in patients with oligospermia and azoospermia. There was no correlation between serum FSH and LH levels. Basal FSH levels and FSH response to LH–RH are always abnormally high when spermatids are not yet formed in the biopsy specimen. There is no correlation evident between LH levels and LH response to LH–RH on the one hand and the stage of spermatogenesis on the other. Prolactin secretion seems to be normal in these patients.

In normal seminal plasma a protein substance was found lowering basal FSH levels without altering those of LH and preferentially decreasing the FSH response to the injection of LH–RH in the castrate and normal rat. The substance may be involved in the regulatory mechanism of FSH secretion.

In the study of the regulation of gonadotrophin secretion, we intend to consider two conditions which are not directly related to steroid secretion or steroid administration: firstly the relationship between spermatogenesis and gonadotrophins and secondly the gonadotrophin inhibiting activity of seminal plasma.

I. RELATIONSHIP BETWEEN GONADOTROPHIN LEVELS AND SPERMATOGENESIS

The study of idiopathic infertility contributes to a better understanding of the relationship between spermatogenesis and FSH secretion and allows us to discuss again the origin of the signal coming from the seminiferous tubules and leading to changes in FSH secretion. So far, there have been three different views regarding this relationship.

1. There is a specific stage of spermatogenesis involved in testicular feedback on FSH: spermatid formation [1] or maturation of spermatozoa [2].
2. There is no correlation of FSH levels with any specific cell stage and the germinal cells do not play any role directly or indirectly in the feedback regulation of FSH secretion [3, 4].
3. There is no feedback signal from any specific stage of spermatogenesis but an inverse correlation exists between FSH levels and the severity of the reduction of germinal cells from spermatogonia to late spermatids [5].

In order to pursue the study of the relationship between spermatogenesis and the gonadotrophins, we have analysed the correlations between basal FSH and LH levels, FSH and LH response to LH-RH injection, sperm count, stage of spermatogenesis and prolactin levels.

1. *Correlation between sperm count and FSH and LH levels*

In 85 cases of oligospermia and azoospermia there was no correlation between serum FSH and LH levels and the sperm count. This present study confirms the data previously reported [4, 1, 3]. Nevertheless this lack of correlation has not been a universal finding. Rosen and Weintraub[6] found an inverse correlation between sperm count and serum FSH concentration in oligospermic subjects. Mauss and Borsch[7] also showed an inverse relationship between the logarithm of sperm count and urinary excretion of FSH.

2. *Correlation between basal gonadotrophin (FSH and LH) levels and response to LH–RH*

In 28 of these subjects, 25 μg of LH–RH was injected intravenously. Response was evaluated by the maximum FSH levels attained during the two hours following LH–RH injection or the cumulative response, calculated from the area under the serum FSH and LH curves during the first 2 h following LH–RH. We found a close correlation between the two parameters and the FSH basal levels (Fig. 1.) Thus all the patients with high basal FSH levels showed higher responses to LH–RH than the normal subjects. Similarly patients with normal FSH levels displayed a normal response to LH–RH except in 2 cases, where there was an abnormally high FSH response to LH–RH. The biopsy stages of these two patients were 5 and 6 respectively. This discrepancy in these two cases could be due to the spontaneous fluctuation of FSH observed in gonadal disorders [8].

A correlation between basal LH levels and the LH response to 25 μg LH–RH was found in the 28 cases studied (Fig. 2).

From these observations we may accept that the basal levels of gonadotrophins is an index of pituitary reserve of gonadotrophin releasable by a constant amount of exogenous LH–RH.

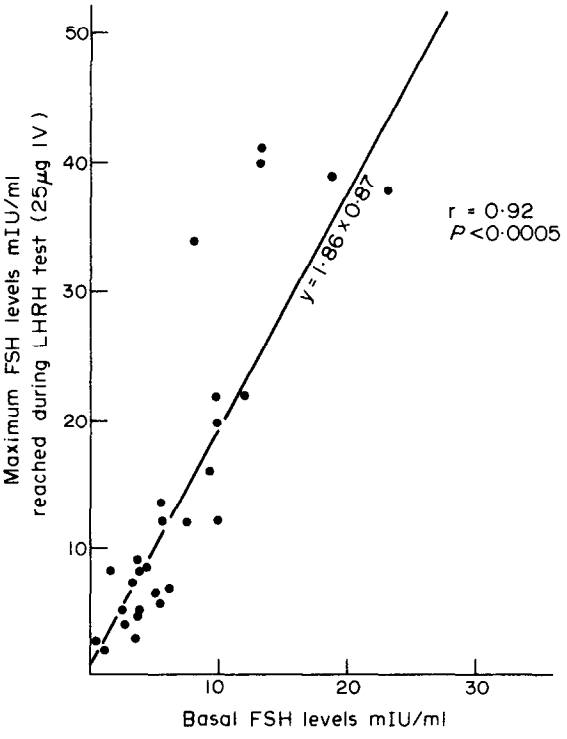

Fig. 1. Relationship between the maximum FSH levels reached during the two first hours following LH–RH (25 μg) injection and basal FSH levels in 29 azoospermic or oligospermic patients.

3. *Correlation between basal FSH and LH levels and stage of spermatogenesis*

In 85 cases of oligospermia and azoospermia studied we found a direct correlation ($r = 0{\cdot}79$) between the basal FSH levels and the stage of spermatogenesis as defined earlier [1] (Fig. 3). It must be pointed out that FSH levels are always abnormally high between stages 0 to 4 *i.e.* when spermatids are not yet formed. But the highest levels of FSH are found when spermatogenesis is arrested at earlier stages or completely absent. As previously reported [1] there is no obvious relationship between LH levels and

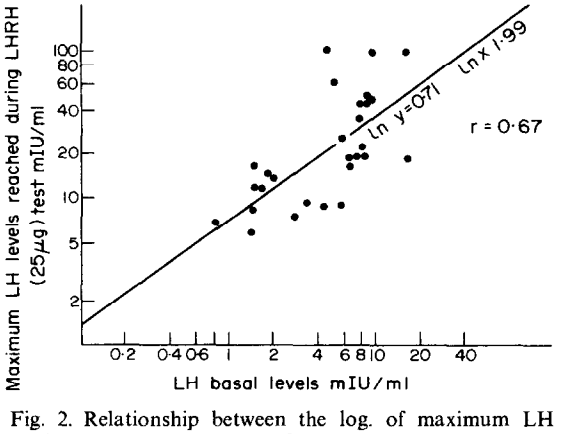

Fig. 2. Relationship between the log. of maximum LH levels reached during the two first hours following LH–RH (25 μg) injection and basal LH levels.

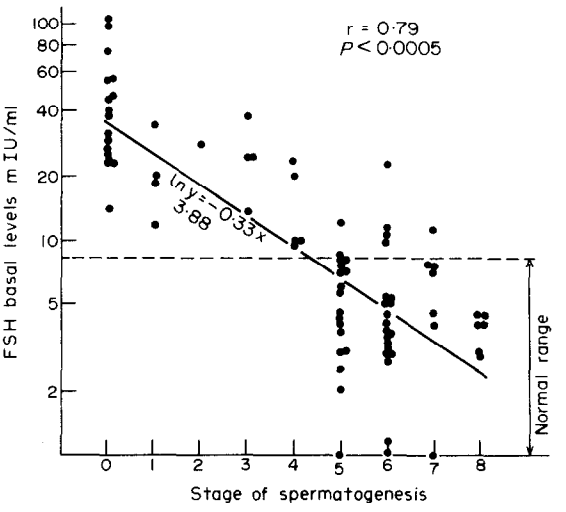

Fig. 3. Correlation between the stage of spermatogenesis observed at the testicular biopsy and basal FSH levels. Stages of spermatogenesis was described previously (Franchimont *et al.*, 1972): Stage 0 = All the seminiferous tubules are hyalinized; Stage 1 = Sertoli cells are the only constituent of the seminiferous epithelium; Stage 2 = In addition to Sertoli cells, some spermatogonia are present; Stage 3 = Sertoli cells, spermatogonia and a few scattered primary spermatocytes are seen; Stage 4 = Maturation is arrested at the primary spermatocyte stage, with great numbers of these cells forming a rather continuous layer around the tubular lumen. This stage corresponds to spermatogenic arrest; Stage 5 = In addition some spermatids are present but spermatozoa are entirely absent; Stage 6 = Maturation is complete with a normal number of spermatids but only some spermatozoa; Stage 7 = All stages are represented in suitable proportions but the number of germ cells is low: the seminiferous epithelium consists of only three or four cell layers; Stage 8 = Spermatogenesis is normal.

LH response to LH–RH on the one hand and the stage of spermatogenesis on the other.

4. *Correlation between prolactin and spermatogenesis*

In 15 of these subjects, 200 μg of TRH were injected and prolactin was assayed in the sera collected 20 and 60 min later. In all cases, prolactin reached its maximum level within the range of ±2 standard deviations of the mean of the normal maximum response: 43·4 ng ± 10·1 (±1 S.D.).

On the basis of the basal FSH levels and the FSH response to LH–RH we may divide azoospermic and oligospermic patients into two groups.

a) Patients with normal FSH levels and normal response to LH–RH.

b) Patients with high FSH levels and response to LH–RH.

All the patients with arrest of spermatogenesis prior to spermatid formation fall in the latter category. This confirms our earlier observation, [1]; nevertheless there is an inverse correlation between basal FSH levels and FSH response to LH–RH on the one hand and the stage of spermatogenesis on the other. Thus the higher the FSH levels or response to LH–RH,

the more depleted is the basal germinal cell population.

To interpret the constant high FSH levels and response to LH RH when spermatids are absent on biopsy, one may postulate that the maturation of spermatids could induce or permit the formation of a factor controlling FSH secretion. Alternatively, spermatids could have no specific function in FSH regulation and reflect only a severe depression of the basal germinal cell population resulting in increased FSH levels.

There is no correlation between basal LH levels and LH response to LH–RH on the one hand and the defect in spermatogenesis judged by sperm count or analysis of testicular biopsy on the other. The TRH induced prolactin release is normal in the azoospermic and oligospermic cases studied.

II. GONADOTROPHIN INHIBITING ACTIVITY OF SEMINAL PLASMA "INHIBIN"

There is ample evidence for the presence of a substance produced by the testis that selectively inhibits the secretion of pituitary FSH. This substance was first suspected by McCullagh in 1932 [9]. More recently, Setchell and Siriranganathji[10] have reported on a thermolabile substance with antigonadotrophic activity in the rete testis fluid of the ram. In the same year we found that the injection of human seminal plasma decreased FSH levels in castrated male rats [11].

Lee *et al.*[12] have shown selective suppression of plasma FSH in sheep by extracts of bovine testis essentially free of testosterone. On the contrary Hodgen *et al.*[13] observed a decrease in LH levels without change in plasma FSH concentrations in castrated rats injected with adult testis homogenate. The observed effect was not due to testosterone in the homogenate and was not produced by the epididymis.

We have extended our own investigation on the existence, nature and specificity of inhibitory action of this possible "inhibin" in seminal plasma of bull and man.

1. *Extraction of semen*

Bull or human semen was centrifuged for 15 min at 3000 rev./min to separate sperm from seminal plasma.

Protein was precipitated by adding absolute ethanol to the fluid to a concentration of 86%. The precipitate was recovered by centrifugation at 4°C, dissolved in distilled water and lyophylised. This fraction is referred to as crude material.

The supernatant was treated with ether–chloroform. This ether soluble fraction was used for steroid analysis and showed no inhibition of either FSH or LH levels in castrated male rats. Three hundred mg. of crude material were subjected to gel filtration on Sephadex G100 (90 × 2·5 cm.) using either 0·05 M Na-acetate buffer pH 4 or 0·05 M phosphate buffer pH 7·4. Fractions of 2·5 cm^3 were collected. All gel filtrations were carried out in a cold room at 4°C.

When crude bovine material is applied to a column of Sephadex G100 at alkaline pH the elution profile shows two peaks, the first an unretarded distinct peak (P 1) and the second a poorly defined peak (P 11). In acidic conditions, there is a distinct separation of two peaks (Ac 1 and Ac 11). Ac 1 is eluted in the void volume whereas Ac 11 is eluted in the included volume (Fig. 4).

2. *Evaluation of biological activity*

The inhibiting effect of different fractions on FSH and LH was determined in castrated adult male rats weighing between 180 and 200 g. Fourteen days after castration animals were injected intraperitoneally with buffer saline (control) or samples. The total dose was administered in 4 injections at 12 h intervals. Four hours after the last injection, the animals were bled and FSH and LH were assayed using NIAMD systems. In five experiments performed with 5 different batches of bull seminal plasma, Ac 1 at the dose of 500 μg injected intraperitoneally showed a slight but not significant decrease in FSH and LH basal levels. On the other hand, Ac 11 at a dose of 200 μg per rat, induced a significant decrease in FSH levels whereas the reduction in LH was inconsistent and

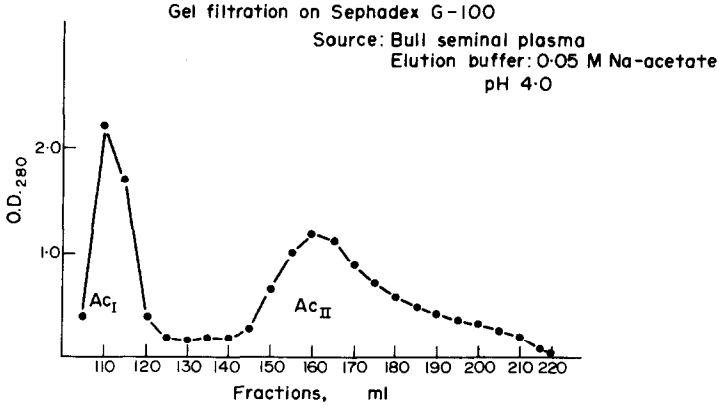

Fig. 4. Elution profile of crude material from bull on Sephadex G 100 using acidic buffer.

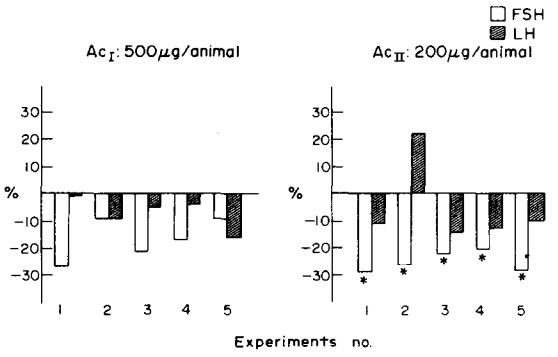

Fig. 5. Decrease of FSH and LH levels in castrated rats treated with the first acidic fraction (Ac 1) and the second acidic fraction (Ac 11) expressed in % compared with control animals (0).

was never significant (Fig. 5). To ascertain the peptide nature of the substance isolated by gel filtration at alkaline pH, it was subjected to pepsin digestion. Digestion was carried out in glycine HCl buffer pH 2 with an enzyme substrate ratio of 1:50 and incubated for 24 h at 37°C. This material was used for treating animals. Pepsin digestion destroyed the inhibiting ability of P 11.

The absence of the steroids: testosterone, progesterone and 17β-estradiol in the material collected in the second peak (Ac 11 or P 11) was confirmed by radioimmunoassay.

3. *Action of the Ac 11 on response to LH–RH*

Fourteen days after castration, animals were treated with 200 μg of Ac 11 injected intraperitoneally as described earlier. One hour after the last injection

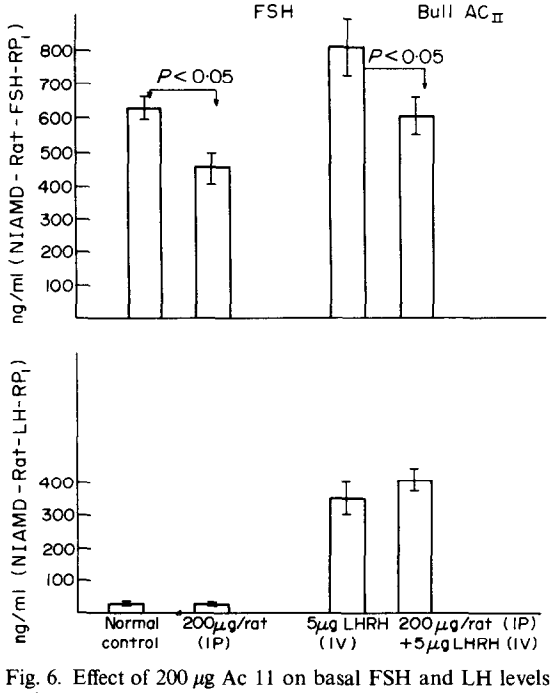

Fig. 6. Effect of 200 μg Ac 11 on basal FSH and LH levels and on the response to 5 μg LH–RH injection in normal rats (mean ± 1 S.E.).

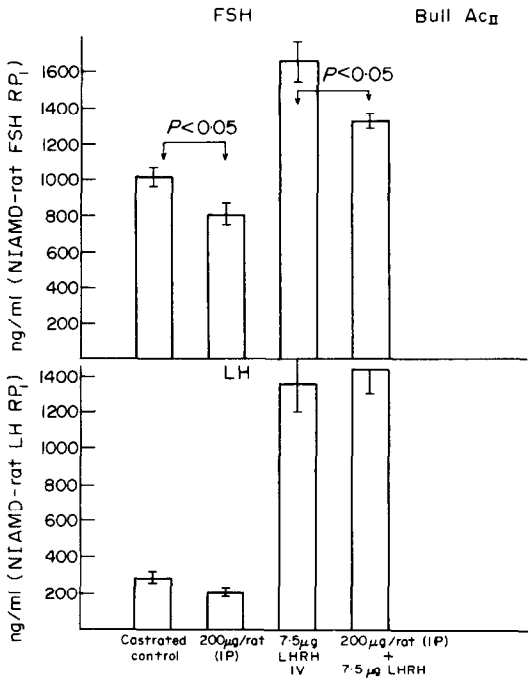

Fig. 7. Effect of 200 μg Ac 11 injected intraperitoneally on basal FSH and LH levels and on the response to 7,5 μg LH–RH in castrated rats (mean ± S.E.).

5 or 7·5 μg of LH–RH diluted in 0·5 ml of NaCl 0·9% was administered *via* the tail vein. Thirty min later the animals were killed. Normal male rats were treated similarly.

Both in normal and castrated rats 200 μg of bull Ac 11 decreased the basal FSH levels without any significant change of LH. When LH–RH was injected, FSH and LH levels increased significantly. The pretreatment of rats with Ac 11 diminished the response of FSH to LH–RH without affecting the LH response (Figs 6, 7).

In a different experiment, Ac 11 samples were injected intravenously one hour before the administration of LH–RH and animals were killed 30 min later. A significant decrease of FSH levels was again observed. The increase of FSH and LH levels induced by LH–RH was diminished by prior treatment with 200 μg of bull Ac 11.

From this initial experiment we may draw the following conclusions:

In human and bull seminal plasma there is a substance(s) which selectively decreases basal FSH levels. This substance appears to be polypeptide in nature.

Injected intraperitoneally it decreases the FSH reponse to LH–RH injection. On the other hand, injected intravenously it produces a decrease in both FSH and LH responses to LH–RH. Why the route of administration changes the nature of response is not yet clear.

CONCLUSIONS

In azoospermia and oligospermia without Leydig cell deficiency, FSH levels are frequently raised and

there is a correlation between the stage of arrest of spermatogenesis and the levels of FSH. A similar correlation does not exist for LH. The precise regulatory mechanism has not yet been established. A protein substance present in seminal plasma lowers basal FSH levels without altering those of LH and preferentially decreases the FSH response to the injection of LH–RH in the castrate and normal rat. This substance may be involved in the regulatory mechanism. An extensive study of it is currently underway.

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