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The role of testicular fluid on blood plasma levels of FSH and LH in the ram (1)

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Summary. The secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) is partly regulated by a non-steroid factor, inhibin, present in the testicular lymph and rete testis fluid (RTF). We were interested to know whether the RTF excreted into the epididymis has a regulatory role on the secretion of FSH and LH.

The remaining rete testis of 6 adult rams hemicastrated 6 to 10 months earlier was cannulated. These rams were castrated either the day after the flow of RTF spontaneously stopped or 15 days after cannulation if the cannula was still functional. Two daily blood samples were taken from 7 days before cannulation to 7 days after castration and circulating levels of FSH, LH and testosterone were measured. Rete testis cannulation did not affect FSH, LH or testosterone plasma levels, although castration induced a sudden decrease in testosterone concentration and a rapid increase in FSH and LH levels.

The present results suggest that the inhibin present in the RTF excreted into the epididymis does not play any role in the regulation of FSH and LH secretion. These hormones could be regulated by the inhibin present in the efferent circulation (lymph or blood) from the testis.

Introduction.

Ovine testicular fluid secreted by the testis into the epididymis has been shown to contain inhibin (Setchell and Sirinathsinghji, 1972 ; Setchell and Jacks, 1974 ; Baker *et al.*, 1976 ; Blanc and Dacheux, 1976 ; Davies *et al.*, 1976 ; Franchimont *et al.*, 1977). Since this substance is known to regulate FSH and LH in the ram (Blanc *et al.*, 1978 ; Cahoreau *et al.*, 1979), we were interested to know whether the RTF has a regulatory role on the level of gonadotrophins in the peripheral blood of the adult ram, or whether, since testicular lymph is known to contain inhibin activity (Eddie *et*

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al., 1977 ; Baker *et al.*, 1978), this role might be relevant to the inhibin secreted by the Sertoli cells into the extratubular compartment of the testis and drained through the lymphatic vessels to the blood stream. Such an hypothesis has been also postulated by Cahoreau *et al.* (1977) and Walton *et al.* (1978). In order to investigate this alternative, the remaining testis of adult hemicastrated rams was cannulated to remove RTF and prevent its reabsorption by the epididymis ; concentrations of FSH, LH and testosterone in the peripheral blood have been compared before and during cannulation as well as after castration. The results are discussed in the present report.

Material and methods.

Animals. — This experiment was carried out at the end of the breeding season (November) on 6 adult Ile-de-France rams, hemicastrated 6 to 10 months previously.

Experimental design. — The testicular fluid (RTF) which would have been secreted into the epididymis through the ductuli efferentes was collected from a cannula inserted into the rete testis at the top of the testis according to the method of Voglmayr *et al.* (1966) modified by Edwards *et al.* (1976). The animals were castrated in the morning 15 days later or on the day after the flow of RTF spontaneously stopped. The RTF was collected at 4 °C on the animal ; its volume and sperm density were checked every 24 hrs.

Blood samples were collected twice a day at 08.30 hr and 17.00 hr by acute jugular venepuncture from 7 days before insertion of the cannula to 7 days after castration. Blood plasma was immediately separated by centrifugation at 1 500 g and stored at — 20 °C until assay.

Hormone assays. — LH, FSH and testosterone were measured in plasma samples by specific radioimmunoassays using the double antibody techniques of Pelletier *et al.* (1968) for LH, Blanc and Poirier (1979) for FSH and Garnier *et al.* (1978) for testosterone. Results are expressed as ng of reference preparations of these hormones per ml of blood plasma. As the levels in morning and evening samples did not differ significantly within animals, results are given as mean daily concentrations of hormones in the plasma.

Analysis of results. — For each hormone, the daily individual results of each of the 6 rams have been pooled (mean $\pm$ s.e.m.) for comparisons of hormonal levels before and after cannulation as well as before and after castration. Statistical analyses were performed by « t » test of paired data.

Results.

a) *Production of RTF and spermatozoa.*

Two rams were castrated when the cannula was still operating on day 15 ; in other animals, flow of testicular fluid stopped on day 6 ($n = 2$), day 11 ($n = 1$) and day 12 ($n = 1$). The fluid outflow, overall mean : 1.32 ± 0.1 ml/h, sperm density : $143 \pm 17 \times 10^6$ spermatozoa (spz)/ml, and daily collected spz : $5.12 \pm 0.38 \times 10^9$,

were fairly variable between animals but quite consistent throughout the period of cannulation for each animal except in one ram in which sperm density and daily collected spermatozoa decreased sharply from day 6 onwards.

b) *Hormonal patterns.*

As shown in figures 1*a*, *b*, *c*, removal of whole testicular fluid secreted by the testis into the ductuli efferentes did not change the peripheral blood plasma concentrations of either FSH, LH or testosterone. Means and standard errors of the mean did not differ significantly before and throughout the period of cannulation. The pattern observed for testosterone, with a general trend for the concentration of this steroid to decrease throughout the experiment, was not related to cannulation but was possibly a function of season.

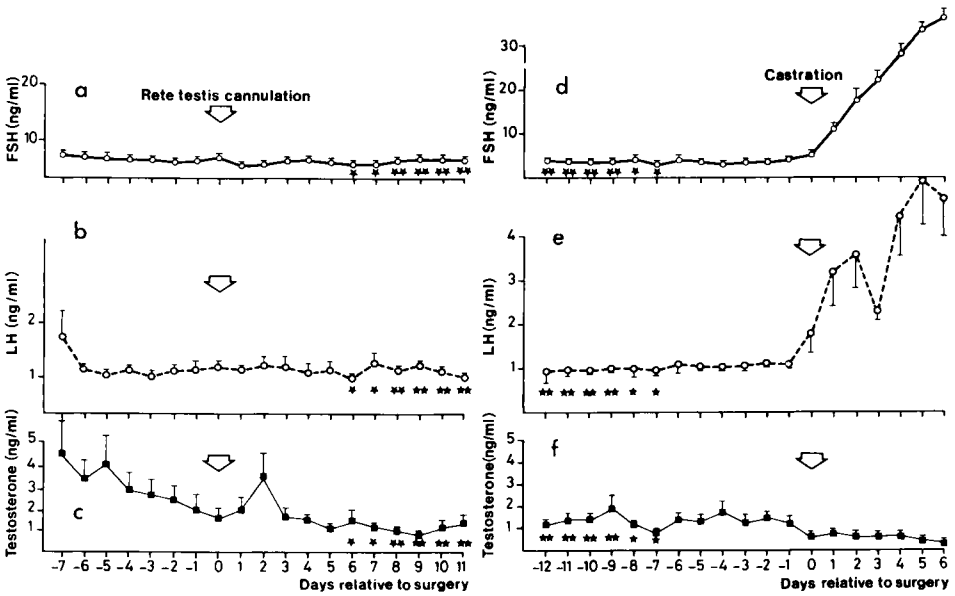

FIG. 1. — *Effect of cannulation of the rete testis* (a, b, c) *and castration* (d, e, f), *on peripheral plasma levels of FSH, LH and testosterone in hemicastrated Ile-de-France rams.*

Results are given as mean ± s.e.m. (n = 6, except for *, n = 5 and **, n = 4).
Reference standard preparations : — FSH : CNRS FSH P26 (14 times NIH-FSH-S3) ; — LH : CNRS LH M3 (1.8 times NIH-LH-S1).

In the same rams, removal of the remaining testis resulted in a significant and sharp increase of blood LH and FSH from the day after castration onwards, ending in a 5-fold and 9-fold increase, respectively, 7 days after orchidectomy, and a small decrease of testosterone : 0.5 versus 1.4 ng/ml after vs before castration (fig. 1*d*, *e*, *f*). On the day of castration, the mean LH plasma level was already higher than before castration due to the rapid increase of the hormone concentration as soon as a few hours after castration.

Discussion.

The experiment reported here was performed to investigate the physiological importance of inhibin contained in RFT for the regulation of gonadotrophins. The effect of diversion of RTF before being reabsorbed in the epididymis on circulating FSH and LH was thought to highlight the role of the material which normally flows through the epididymis.

As did Walton *et al.* (1978), we carried out the experiment in hemicastrated rams since it is surgically more successful to cannulate the remaining rete testis of such animals instead of both organs of normal rams. Animals were used a long time after hemicastration, when compensatory testicular hypertrophy was completely established (Hochereau-de Reviers *et al.*, 1976). This would explain the elevated sperm density in RTF observed in the present experiment ($143 \pm 17 \times 10^6$ spz./ml) which is higher than that reported for entire Ile-de-France rams in the breeding season ($113 \pm 8 \times 10^6$ spz./ml) (Dacheux *et al.*, 1977). With a similar flow rate of fluid in both groups of animals, this results in a higher number of spermatozoa collected per testis in hemicastrated as compared to normal rams : 5.1 (present results) versus 3.5×10^9 spz./day (Dacheux *et al.*, 1977). These figures are surprisingly similar to 5.7 ± 0.5 and $3.9 \pm 0.3 \times 10^9$ spz./day calculated from quantitative histological data used to estimate the daily production of round spermatids per testis in the entire and hemicastrated ram during the breeding season (Hochereau-de Reviers *et al.*, 1976). This suggests that both techniques, testicular cannulation as well as quantitative histology, are equally reliable when used to estimate daily sperm production in the ram. It also shows that testicular cannulation, when operated properly, does not interfere with testicular sperm production at least during several days after surgery (Voglmayr *et al.*, 1967 ; Walton *et al.*, 1978 : in which the mean daily sperm production of hemicastrated rams, $4.6 \pm 0.34 \times 10^9$, is similar to that observed in the present work ; Cahoreau *et al.*, 1979).

Sperm production of the rams used in this experiment was indicative of active spermatogenesis. Since this process is probably related to the inhibin content of RTF (Voglmayr *et al.*, 1976), it is clear that hemicastrated rams were appropriate to study the effect of removal of testicular fluid on the circulating gonadotrophins.

It is evident from our results that cannulation of the rete testis during the breeding season did not change the levels of LH and FSH in the peripheral blood plasma of Ile-de-France rams whereas orchidectomy induced an increase of the same parameters. This suggests that RTF may not be the major route of secretion of the inhibin which is biologically active in the regulation of gonadotrophins. Similar results were obtained by Walton *et al.* (1978) after testicular cannulation of mature Clun Forest rams between July and November, which is earlier in the breeding season than in the present experiment. These authors concluded that « the inhibin in RFT may not contribute significantly to the overall feedback control of FSH in rams during the autumn ». These results agree with those of Setchell *et al.* (1977) and Davies *et al.* (1978) in the rat where it was shown that ligation of efferent ducts of the testis, which prevents the fluid secreted by the gonad from flowing into the epididymis, was without immediate effect on FSH secretion. FSH began to rise only after a delay when the tubules decreased

in size as a consequence of a disorganization of the germinal epithelium (Setchell *et al.*, 1977), resulting in a decrease in production of inhibin (Voglmayr *et al.*, 1976).

Inhibin must enter, at least partly, into the blood and/or the lymphatic flow independently of entering RTF. This argument is supported by the presence of inhibin activity in ovine testicular lymph (Eddie *et al.*, 1977 ; Baker *et al.*, 1978). Thus, in normal animals inhibin would be secreted partly into the lumen of the tubules reaching the rete testis and epididymis (Le Lannou and Chambon, 1977) and partly directly from the Sertoli cells into the lymphatic and capillary network through the basement membrane of the seminiferous tubules. This is consistent with the structure of the testis barrier in the tubules as Sertoli cells are present both in the adluminal and external compartments of the tubules (Dym and Fawcett, 1970). Thus, in addition to steroids, only inhibin secreted in the lymph and blood may contribute to the feed-back control of FSH and LH.

The physiological role of the RTF which flows through the epididymis remains to be carefully studied. Could it be related to the maturation process of sperm cells in the epididymis ? This needs further research.

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Résumé. La régulation de la sécrétion des hormones folliculo-stimulante (FSH) et lutéinisante (LH) est en partie assurée par un facteur non stéroïdien, l'inhibine, présent dans la lymphe efférente testiculaire et dans le liquide de « rete testis » (RTF). Aussi était-il intéressant de savoir si le RTF excrété dans l'épididyme avait un rôle régulateur sur la sécrétion de FSH et de LH.

Une canulation du « rete testis » a été pratiquée sur 6 béliers adultes hémicastrés depuis 6 à 10 mois. Ces béliers ont été castrés, soit le jour suivant l'arrêt spontané de l'écoulement du RTF, soit 15 jours après la canulation si celle-ci était encore fonctionnelle. Des prélèvements sanguins (2 par jour) ont été effectués du 7e jour avant la canulation au 7e jour après la castration en vue du dosage RIA des hormones circulantes. La canulation du « rete testis » n'affecte pas les taux plasmatiques de FSH, LH et testosterone. Par contre, la castration provoque une diminution immédiate de testostérone et une augmentation rapide des concentrations plasmatiques de FSH et de LH.

Ces résultats suggèrent que l'inhibine présente dans le RTF et excrétée dans l'épididyme n'intervient pas dans la régulation de la sécrétion de FSH et de LH. Ce rôle régulateur pourrait être assuré par l'inhibine présente dans la circulation efférente (lymphatique ou sanguine) du testicule.

References

BAKER H. W. G., BREMNER W. J., BURGER H. G., de KRETSER D. M., DULMANIS A., EDDIE L. W., HUDSON B., KEOGH E. J., LEE V. W. K., RENNIE G. C., 1976. Testicular control of follicle stimulating hormone secretion. *Rec. Progr. Horm. Res.*, **32**, 429-469.

BAKER H. W. G., BURGER H. G., de KRETSER D. M., EDDIE L. W., HIGGINSON R. E., HUDSON B., LEE V. W. K., 1978. Studies on purification of inhibin from ovine testicular secretions using an « in vitro » bioassay. *Int. J. Androl.*, Suppl. **2**, 115-124.

BLANC M. R., CAHOREAU C., COUROT M., DACHEUX J. L., HOCHEREAU-de REVIERS M. T.,

PISSELET C., 1978. Plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) suppression in the cryptochid ram by a nonsteroid factor (inhibin) from ram rete testis fluid. *Int. J. Androl.*, Suppl. **2,** 139-146.

BLANC M. R., DACHEUX J. L., 1976. Existence of inhibin activity in the ram rete testis fluid : effect of physiological doses on plasma LH and FSH. *I. R. C. S. Med. Sci.*, **4,** 460.

BLANC M. R., POIRIER J. C., 1979. A new homologous radioimmunoassay for ovine follicle stimulating hormone : development and characterization. *Ann. Biol. anim. Bioch. Biophys.*, **19,** 1011-1026.

CAHOREAU C., LACROIX A., BLANC M. R., COUROT M., DACHEUX J. L., HOCHEREAU-de REVIERS M. T., PISSELET C., TERQUI M., 1977. Influence des sécrétions exocrines et endocrines du testicule sur les niveaux plasmatiques de FSH et LH chez le bélier. *J. Physiol., Paris,* **73,** 94A (abstr.).

CAHOREAU C., BLANC M. R., DACHEUX J. L., PISSELET C., COUROT M., 1979. Inhibin activity in ram rete testis fluid : depression of plasma follitropin (FSH) and lutropin (LH) in the castrate and cryptorchid ram. *J. Reprod. Fert.*, (in press).

DACHEUX J. L., PISSELET C., BLANC M., HOCHEREAU-de REVIERS M. T., COUROT M., 1977. Variations saisonnières des sécrétions exocrines (fluide et spermatozoïdes) chez le bélier adulte (Ile-de-France). *J. Physiol., Paris,* **73,** 99A (abstr.).

DAVIES R. V., MAIN S. J., YOUNG M. G. W. L., SETCHELL B. P., 1976. Bioassay of inhibin-like activity in rete testis fluid and its partial purification. *J. Endocr.*, **68,** 26P (abstr.).

DAVIES R. V., MAIN S. J., SETCHELL B. P., 1978. Inhibin : Evidence for its existence, methods of bioassay and nature of the active material. *Int. J. Androl.*, Suppl. **2,** 102-114.

DYM M., FAWCETT D. W., 1970. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol. Reprod,* **3,** 308-326.

EDDIE L. W., BAKER H. W. G., HIGGINSON R. E., HUDSON B., KEOGH E. J., de KRETSER D. M., BURGER H. G., 1977. An « in vitro » bioassay for inhibin. *Endocrinology,* **100,** Suppl., Abstr. n° 268.

EDWARDS E. M., DACHEUX J. L., WAITES G. M. H., 1976. Effects of α-chlorohydrin on the metabolism of testicular and epididymal spermatozoa of rams. *J. Reprod. Fert.*, **48,** 265-270.

FRANCHIMONT P., CHARI S., HAZEE-HAGELSTEIN M. T., DEBRUCHE M. L., DURAISWAMI S., 1977. Evidence for the existence of inhibin, 253-270. In TROEN P., NANKIN H. R., *The testis in normal and infertile men.* Raven Press, N. Y.

GARNIER D. H., COTTA Y., TERQUI M., 1978. Androgen radioimmunoassay in the ram : results of direct plasma testosterone and dehydroepiandrosterone measurement and physiological evaluation. *Ann. Biol. anim. Bioch. Biophys.*, **18,** 265-281.

HOCHEREAU-de REVIERS M. T., LOIR M., PELLETIER J., 1976. Seasonal variations in the response of the testis and LH levels to hemicastration of adult rams. *J. Reprod. Fert.*, **46,** 203-209.

LE LANNOU D., CHAMBON Y., 1977. Présence dans l'épididyme d'un facteur abaissant fortement le taux sanguin de FSH chez le rat. *C. R. Soc. Biol.*, **171,** 636-638.

PELLETIER J., KANN G., DOLAIS J., ROSSELIN G., 1968. Dosage radioimmunologique de l'hormone lutéinisante plasmatique chez le mouton. Mise au point de la technique de dosage. *C. R. Acad. Sci., Paris, Sér. D,* **266,** 2291-2294.

SETCHELL B. P., JACKS F., 1974. Inhibin-like activity in rete testis fluid. *J. Endocr.*, **62,** 675-676.

SETCHELL B. P., MAIN S. J., DAVIES R. V., 1977. Effect of ligation of the efferent ducts of the testis on serum gonadotrophins and testosterone in rats. *J. Endocr.*, **72,** 13P-14P (abstr.).

SETCHELL B. P., SIRINATHSINGHJI D. J., 1972. Antigonadotrophic activity in rete testis fluid. A possible « inhibin ». *J. Endocr.* **53,** LX-LXI (abstr.).

VOGLMAYR J. K., WAITES G. M. H., SETCHELL B. P., 1966. Studies on spermatozoa and fluid collected directly from the testis of the conscious ram. *Nature,* **210,** 861-863.

VOGLMAYR, J. K., SCOTT T. W., SETCHELL B. P., WAITES G. M. H., 1967. Metabolism of testicular spermatozoa and characteristics of testicular fluid collected from conscious ram. *J. Reprod. Fert.*, **14,** 87-99.

VOGLMAYR J. K., ROBERSON C., BARTKE A., 1976. Effects of local testicular heating and rete testis fluid on plasma FSH and testosterone levels in male rats. *J. Reprod. Fert.*, **46,** 497-498.

WALTON J. S., EVINS J. D., WAITES G. M. H., 1978. Effect of chronic removal of testicular fluid on the release of FSH in hemicastrated rams. *J. Endocr.*, **77,** 421-422.