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# Catecholamine Levels and Activity of Monoamine Oxidase in Some Hypothalamic Structures and in the Pineal Gland of Sheep after Administration of FSH

B. PÁSTOROVÁ, J. VÁRADY

*Department of Comparative Physiology, University of Veterinary Medicine, Košice, Slovak Republic*

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## Summary

**The influence of hormonal preparations of FSH in a dose of 24 mg (480 IU) on levels of catecholamine (dopamine, norepinephrine and epinephrine) and the activity of their degradation enzyme monoamine oxidase (MAO) in the hypothalamic regions regulating the reproductive system of sheep (area preoptica, eminentia mediana, corpus mamillare) and pineal gland were investigated in the oestrous period employing radiochemical methods. The administration of FSH resulted in significant ($p<0.001$) increases of dopamine levels in the area preoptica and corpus mamillare of the hypothalamus of sheep as compared to control groups with synchronized oestrus. Hormonal stimulation with FSH increased the levels of hypothalamic norepinephrine in the areas studied and these differences were significant in the eminentia mediana ($p<0.05$) and corpus mamillare ($p<0.05$). Significant ($p<0.001$) changes in epinephrine levels were found in the corpus mamillare and area preoptica ($p<0.05$). Our results indicate that the administration of FSH caused the most pronounced decrease of MAO activity in corpus mamillare ($p<0.001$). The pineal gland reacted to the hormonal preparation by decreased levels of norepinephrine and dopamine ($p<0.001$) and by an increase in MAO activity ($p<0.01$). We suggest that FSH administration affects catecholamine levels and the activity of monoamine oxidase in the studied areas of the brain of sheep by means of a feedback mechanism.**

**Key words**

Catecholamines – Monoamine oxidase – FSH superovulation – Hypothalamus – Pineal gland – Sheep

## Introduction

Hormonal preparations generally used for the induction of superovulation in farm animals affect steroidogenesis of ovaries and influence hypothalamic nuclei and their gonadotropic receptors through a feedback mechanism (Smolich *et al.* 1979, Deaver and Dailey, 1983). High concentrations of circulating oestrogens act specifically upon adrenergic receptors and affect the levels and metabolism of catecholamines in the central and peripheral adrenergic systems (Fernandez-Pardal *et al.* 1986, Pástorová *et al.* 1992, 1994). Simultaneously with changes in the metabolism of catecholamines, some changes in activities of monoamine metabolism enzymes were recorded after hormonal treatment (Chevillard *et al.* 1981). Monoamine oxidase is an enzyme which plays an important role in the degradation of catecholamines by oxidative deamination and participates in the regulation of the functionally active pool of monoaminergic neurotransmitters in the nervous tissue. Some authors (Saavedra *et al.* 1984, Miyake *et al.* 1987) discovered that oestrogens also modify the enzymatic activity of degradation enzymes of catecholamines – such as MAO and catechol-O-methyltransferase (COMT) – in the hypothalamus and striate region.

In view of the sporadic available information about the effect of superovulation preparations on the catecholaminergic system of the hypothalamus and

pineal (Smolich *et al.* 1979, Pástorová *et al.* 1994), our studies were aimed at the investigation of changes in catecholamines and their degradation enzyme MAO in the regions which regulate the reproductive system of sheep after administration of FSH.

## Material and Methods

In our study we used 20 sheep of Slovak merino breed, age 3–4 years, mean body weight 42±3.1 kg in their oestric period (September – October). Sheep were fed two-times daily standard melasse feed with vitamin additives. The oestrus of all sheep was synchronized with intravaginal polyurethane sponges (Agelin Spofa, Prague) containing 20 mg chlorsuperlutin which was instilled for a period of 13 days. The first group (10 animals) served as controls. After completed synchronization of the oestrus, the sheep of second group (n=10) were hormonally stimulated by the administration FSH *ad usum vet.* (Spofa, Prague). FSH was administered to sheep three-times daily for 2 days in overall doses of 24 mg (480 IU). The animals were slaughtered 104 hours after the first dose of FSH.

We rapidly removed the brain which was segmented by segmental analysis according to Welento *et al.* (1969) and samples were taken from the median eminence, preoptic area (bilaterally) and corpus mamillare. Additional samples were obtained from the pineal gland. Tissues were immersed into liquid nitrogen where they were stored in the frozen state until further processing. Samples for radioenzymatic determination of catecholamines were homogenized in microhomogenizers in cooled $HClO_4$ (0.4 $mol.l^{-1}$) with addition of reduced glutathione (0.05 $ml.l^{-1}$) at 1 $\mu$l per 1 ml tissue, and they were centrifuged at 15 000 x *g* at 0 °C for 30 min. Catecholamines were determined by the radioenzymatic method according to Johnson *et al.* (1980) in 50 $\mu$l plasma (in parallel samples). The radioactivity of catecholamine derivatives was measured on a scintillating spectrometer Packard Tri Carb in a $^3H$ channel. The results are expressed in catecholamine nmol.mg $proteins^{-1}$. Proteins were determined in identical homogenate tissues according to Lowry *et al.* (1951). Due to higher concentrations of catecholamines in the brain, the tissue supernatants were diluted with redistilled water in the ratio 1:20. The coefficient of the methodical variation calculated from 10 repetitions of one sample was 4.2 % for norepinephrine (NE) and 4.1 % for dopamine (DA).

For the determination of MAO, the tissue was homogenized in saccharose (0.25 $ml.l^{-1}$) and the radiochemical method according to Wurtman and Axelrod (1963) was used. $^{14}C$-5-(hydroxy)-tryptamine (Amersham, England) with a specific activity $18.5x10^{-7}$ $Bq.nmol^{-1}$ in a dose of 6.25 nmol per sample was used as a substrate. The substrate is specific for the determination of MAO A and MAO B forms. The activity of MAO was measured using a Packard-Tri Carb scintillating spectrometer in the $^{14}C$ channel. Proteins were determined in the same tissue homogenates. The results were statistically processed by the non-paired t-test and are given as means±S.E.M. in nmol $product.min^{-1}.mg\ proteins^{-1}$.

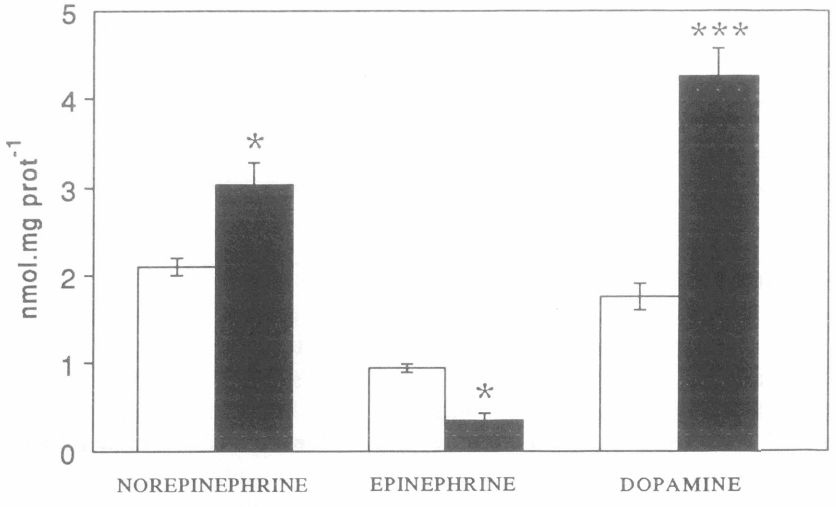


**Fig 1**
*The effect of oestrus synchronization and hormonal stimulation (24 mg FSH) on the levels of catecholamines (norepinephrine, dopamine and epinephrine) in the area preoptica. The results are expressed in nmol.mg protein$^{-1}$ (means±S.E.M.). Open columns represent the control group with synchronized oestrus whereas full columns indicate the group with synchronized oestrus and stimulated by administration of FSH (24 mg). Significant differences from controls: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.*

## Results

After hormonal stimulation of the sheep ovaries with 24 mg FSH (480 IU), a significant increase of norepinephrine levels ($p<0.01$) in area preoptica of the hypothalamus of sheep (Fig. 1) was observed in comparison with the control group. The most marked changes in the concentrations of dopamine ($p<0.001$) after FSH application (from 1.74±0.15 to 4.25±0.31 nmol.mg $protein^{-1}$) were recorded in area preoptica. The levels of epinephrine (EPI) in area preoptica were decreased ($p<0.05$) after hormone administration. In

the median eminence of the sheep hypothalamus (Fig. 2), increased concentrations of NE ($p<0.05$) and EPI ($p<0.01$) were observed after hormone administration. Dopamine in the eminentia mediana exhibited an insignificant increase when compared with the control group. Significant increases in EPI ($p<0.001$), NE ($p<0.05$) and DA concentrations ($p<0.01$) were detected in the corpus mamillare of the sheep hypothalamus treated with 24 mg FSH (Fig. 3).

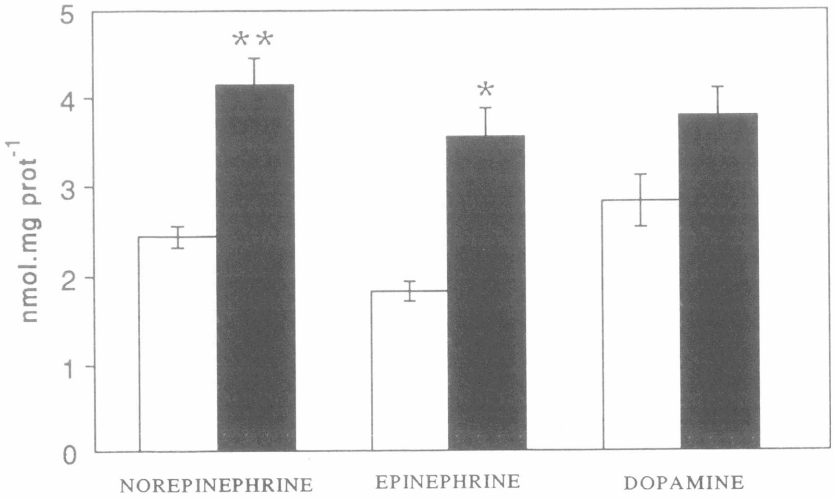


**Fig. 2**
*The effect of oestrus synchronization and hormonal stimulation (24 mg FSH) on the catecholamine levels in the eminentia mediana of sheep. For other details see Fig. 1.*

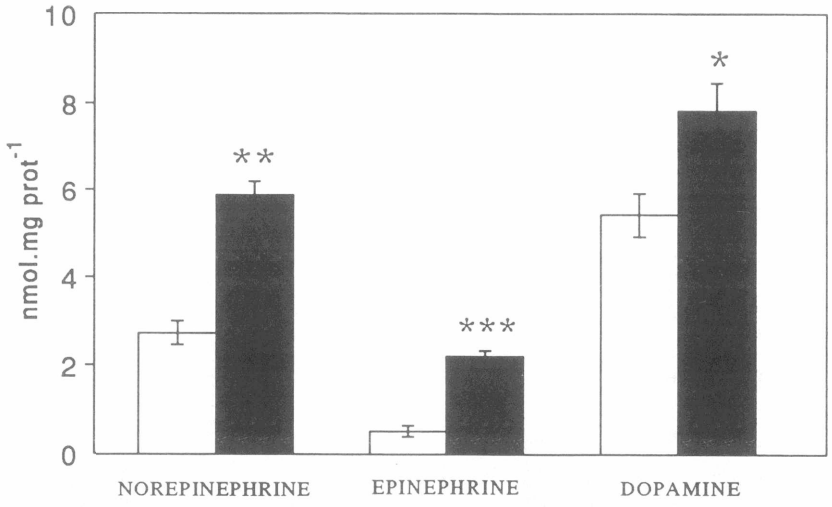


**Fig. 3**
*The effect of oestrus synchronization and hormonal stimulation (24 mg FSH) on the catecholamine levels in the corpus mamillare of sheep. For other details see Fig 1.*

FSH did not alter the activity of the degrading enzyme of catecholamines (MAO) in the preoptic area (Fig. 5). In the corpus mamillare, a decrease in MAO activity occurred after FSH administration ($p<0.001$). In the pineal gland (Fig. 5) a significant increase of MAO activity ($p<0.05$) and decreased NE and DA levels were found ($p<0.001$) (Fig. 4).

## Discussion

The pituitary FSH hormone in redundant amounts induced luteolysis 48 hours after its administration, after which polyovulatory estrus followed (Moor *et al.* 1985, Schiewe *et al.* 1991). At present, most authors (Donnelly and Dailey 1991, Driancourt and Fry 1992) prefer FSH preparations to serum gonadotropins (PMSG) in biotechnically directed reproduction because FSH is a better regulator of the superovulatory process. This ability is due to its short half-life in the organism, and the more stable gonadotropic effect of FSH (Moor *et al.* 1985), although it has to be given several times daily. Fernandez-Pardal *et al.* (1986) have found changes in the activity of monoamine oxidase and catecholamine levels in the ovaries and uterus in ovariectomized HCG- and LH-treated rats, and an increase in the levels of cAMP, which they correlated with an increase in steroidogenesis after hormonal stimulation. The

hormonal preparations used for inducing superovulation of farm animals influence the catecholaminergic system of the hypothalamus and its controlling centres of reproduction (Smolich *et al.* 1979, Pástorová *et al.* 1992, 1994). Schiewe *et al.* (1991) found a 8 to 10 fold increase in 17$\alpha$-oestradiol with a peak at 24–36 h after FSH administration. The high levels of oestrogens act on adrenergic receptors and influence both the function and levels of catecholamines in tissues and plasma (Pástorová and Várady 1994, Miyake *et al.* 1987). Pilotte *et al.* (1982) and Tobias *et al.* (1983) found a reduced norepinephrine turnover and subsequent LH decrease in blood plasma after administration of oestrogens to ovariectomized rats, although the activity of tyrosine hydroxylase and turnover of dopamine in the hypothalamus increased. In comparison with the above mentioned reports, we observed a significant increase in dopamine ($p<0.05$) and norepinephrine ($p<0.01$) levels after FSH administration in area preoptica of the sheep hypothalamus, but the concentration of epinephrine was decreased ($p<0.05$).

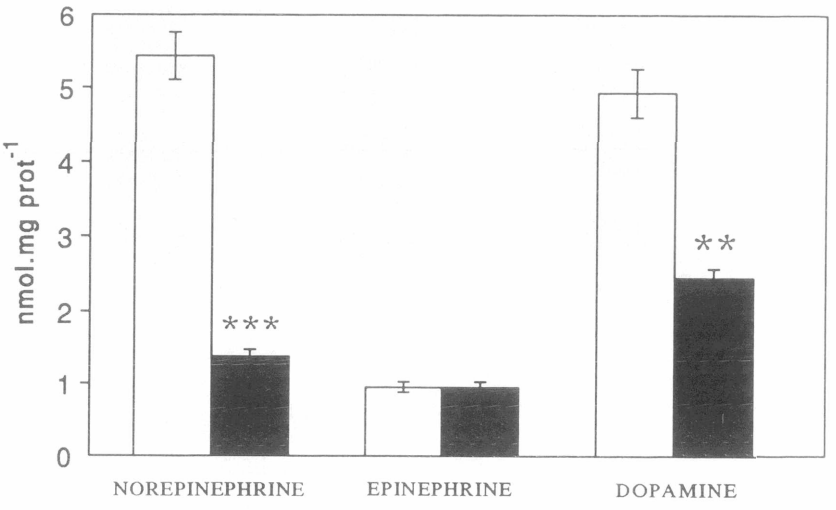


**Fig. 4**
*The influence of hormonal stimulation (24 mg FSH) on the catecholamine levels in the pineal gland of sheep. For other details see Fig 1.*

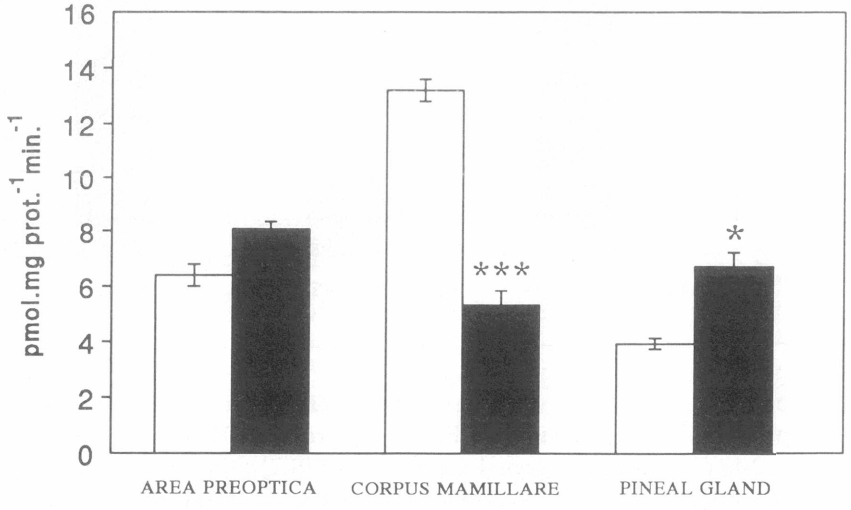


**Fig. 5**
*The effects of oestrus synchronization and hormonal stimulation (24 mg FSH) on activity of monoamine oxidase in area preoptica, corpus mamillare and pineal gland of sheep. Results are expressed in nmol.mg protein$^{-1}$.min$^{-1}$. For other details see Fig 1.*

The most pronounced function in the hypothalamus is ascribed to dopamine occurring in tuberoinfundibular neurones (TIDA) which show cyclic changes of activity during the oestrous cycle of animals (Pilotte *et al.* 1982). These neurones are involved in coordination of hypothalamic control of secretion of three gonadotropic hormones (prolactin, FSH and LH). Dopamine is released by neurons located in eminentia mediana into the portal blood and acts as an important factor of prolactin secretion, probably through the receptors present in lactotropic cells of the hypophysis (Gottschall and Meites 1987). Our results indicate significant increase in norepinephrine ($p<0.05$) and epinephrine ($p<0.01$) levels and an insignificant increase in dopamine levels in the median eminence after hormone stimulation.

Hypothalamic regions participating in the regulation of reproductive functions of sheep also include the mamillary area which harbour the adrenergic nervous terminals involved in the regulation and secretion of gonadotropic hormones. The corpus mamillare of sheep showed a significant increase of norepinephrine ($p<0.01$), epinephrine ($p<0.001$) and dopamine ($p<0.05$) levels and a significant decrease of MAO activity ($p<0.001$) after hormone administration, which were found to correlate. The turnover and actual levels of catecholamines in the nerve tissue depend on more factors such as storage and uptake, transneural flux and interaction with autoreceptors (Saavedra *et al.* 1984). Alterations of some of these factors with hyperoestrogenisation leads to changes in the concentration and functions of catecholamines in the nervous tissue.

Monoamine oxidase is responsible for intraneuronal metabolism of monoamines and regulation of their active pool in the nervous system. It follows from our previous results (Pástorová *et al.* 1992, 1994, 1995) that hormonal preparations (PMSG, HCG and FSH) influence MAO activity in both the hypothalamus and hypophysis of sheep. Other authors (Chevilard *et al.* 1981, Saavedra *et al.* 1984) have found that gonadal steroids change the activity of monoamine oxidase and catechol-O-methyltransferase in different parts of the rat brain. Our results indicate that the activity of MAO in the preoptic area of the sheep hypothalamus is not altered following FSH administration. The most pronounced changes in MAO activity ($p<0.001$) were observed in the corpus mamillare where a significant increase in catecholamines occurred.

In the pineal gland, catecholamines participate in the regulation or modulation of melatonin secretion. The adrenergic neurones increase their activity in darkness, when the synthesis of NE is elevated. Norepinephrine acts on alpha- and beta-receptors of pinealocytes and stimulates the activity of N-acetyltransferase (NAT) – the enzyme synthesizing melatonin (Seltzer *et al.* 1992). Our experiments detected increased activity of monoamine oxidase in the pineal gland ($p<0.05$) after administration of FSH which is consistent with the decrease of norepinephrine and dopamine.

The administration of FSH has an opposite effect in the hypothalamus than on the pineal gland, in which it causes a decrease in catecholamine levels and an increase in MAO activity From our previous work (Pástorová *et al.* 1992, 1994) it may by suggested that FSH markedly influences the activity of MAO in the uterus and in the centres controlling reproductive functions of sheep. We propose that the given changes in MAO activity in the corpus mamillare and pineal gland of sheep are associated with observed changes in the levels of catecholamines related to steroid alterations (Chevillard *et al.* 1981) after administration of the gonadotropic hormone.

## References


CHEVILLARD C., BARDEN N., SAAVEDRA J.M.: Estradiol treatment decreases type A and increases type B monoamine oxidase in specific brain stem areas and cerebellum of ovariectomized rats. *Brain. Res.* **222**: 177–181, 1981.

DEAVER D.R., DAILEY R.A.: Effects of ovarian secretion and dopamine on secretion of luteinizing hormone and prolactin in ewes. *J. Anim. Sci.* **57**: 978–984, 1983.

DONNELLY P.J., DAILEY R.A.: Effect of dopamine, norepinephrine and serotonin on secretion of luteinizing hormone, follicle-stimulating hormone and prolactin in ovariectomized, pituitary stalk-transected ewes. *Domest. Anim. Endocrinol.* **8**: 87–98, 1991.

DRIANCOURT M.A., FRY R.C.: Effect of superovulation with FSH or PMSG on growth and maturation of the ovulatory follicles in sheep. *Anim. Reprod. Sci.* **27**: 279–292, 1992.

FERNANDEZ-PARDAL J., GIMENO M.F., GIMENO A.L.: Catecholamines in sow graafian follicles at proestrus and at diestrus. *Biol. Reprod.* **34**: 439–455, 1986.

GOTTSCHALL P.E., MEITES J.: Bromocryptine prevents the decline in tuberoinfundibular release of dopamine after removal of chronic estrogen treatment. *Proc. Soc. Exp. Biol. Med.* **186**: 150–156, 1987.

JOHNSON G.A., KUPIECKI R.M., BAKER C.A.: Single isotope derivate radioenzymatic methods in the measurement of catecholamines. *Metabolism* **29**(Suppl. 1): 1106–1113, 1980.

LOWRY O., ROSENBROUGH A., FARR R., RANDAL R.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **194**: 265–275, 1951.

MIYAKE A., NISHIZAKI T., OHTSUKA S., YOSHIMOTO Y., AONO T., TANIZAWA O.: Absence of effect of estrogen on dopamine, norepinephrine and beta-endorphin-like activity in human plasma. *Endocrinol. Jpn.* **34**: 789–792, 1987.

MOOR R.M., OSBORN J.C., CROSBY I.M.: Gonadotrophin-induced abnormalities in sheep oocytes after superovulation. *J. Reprod. Fertil.* **74**: 167–172, 1985.

PÁSTOROVÁ B., VÁRADY J.: Catecholamine in estrus levels in sheep during superovulation (in Slovak). *Vet. Med. (Praha)* **37**: 101–107, 1992.

PÁSTOROVÁ B., VÁRADY J., KOZÁČKOVÁ M.: The increase of catecholamine levels and activity of monoamino oxidase in the hypophysis of ewes following hormonal stimulation with serum gonadotrophin. *Homeostasis* **33:** 256–261, 1992.

PÁSTOROVÁ B., STANÍKOVÁ A., RAJTOVÁ V., VÁRADY J.: Changes in some biochemical and histological characteristics in the hypothalamus of hormonally stimulated sheep. *Int. J. Anim. Sci.* **9:** 1–8, 1994.

PÁSTOROVÁ B., HALAGAN J. STANÍKOVÁ A., MOLNÁROVÁ M., VÁRADY J., ELIÁŠ V.: Catecholamine levels and activity of monoamine oxidase in estrus ewes after treatment with PMSG and antisergone. *Physiol. Res.* **44:** 20P, 1995.

PILOTTE N.S., BURT D.R., BARRACLOUGH C.A.: Ovarian steroids modulate the release dopamine into hypophysial portal blood and density of anterior pituitary [$^3$H]spiperone-binding sites of ovariectomized rats. *Endocrinology* **114:** 2306–2311, 1982.

SAAVEDRA J.M., CHEVILLARD C., BISSERBE J.C., BARDEN N.: Estradiol increases dopamine turnover in intermediate and posterior pituitary lobes of ovariectomized rats. *Cell. Mol. Neurobiol.* **4:** 397–402, 1984.

SCHIEWE M.C., FITZ T.A., BROWN J.L., STUART L.D., WILD D.E.: Relationship of oestrous synchronization method, circulating hormones, luteinizing hormone and prostaglandin $F_{2\alpha}$ receptors and luteal progesterone concentration to premature luteal regression in superovulated sheep. *J. Reprod. Fert.* **93:** 19–30, 1991.

SELTZER A., VISWANATHAN M., SAAVEDRA J.M.: Melatonin-binding sites in brain and caudal arteries of the female rat during estrous cycle and after estrogen administration. *Endocrinology* **130:** 1896–1902, 1992.

SMOLICH T.D., SCHAWER G., WEBER G., BUSH W.: Morfokinetische Wirkung von PMSG und Sexualsteroiden auf Gleider der Zerebro-hypophysegonadalen Achse anestrischer Schafe. *Arch. Exp. Vet. Med. (Leipzig)* **33:** 141–149, 1979.

TOBIAS H., CARR L.A., VOOGT J.L.: Catecholamine mechanisms in the feedback effects of oestradiol benzoate on the release LH and prolactin. *Proc. Soc. Exp. Biol. Med.* **174:** 284–290, 1983.

WURTMAN J., AXELROD J.: A sensitive and specific assay for estimation of monoamine oxidase. *Biochem. Pharmacol.* **12:** 1439–1441, 1963.

WELENTO J.S., SZTEYN S., MILART T.: Observation on the stereotaxic configuration of the hypothalamus nuclei in sheep. *Anat. Anz.* **124:** 1–30, 1969.

**Reprint Requests**

B. Pástorová, Department of Physiology, University of Veterinary Medicine, Komenského 73, Košice, Slovak Republic.