ROLES OF MONOAMINES ON CENTRAL REGULATION OF THE PITUITARY LUTEOTROPHIC COMPLEX IN THE GOLDEN HAMSTER

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Summary

It is possible to suppress endogenous release of FSH component of the pituitary luteotrophic complex required for progesterone secretion and blastocyst implantation in the post coitus golden hamster by daily injection of 10–15 *mg/kg of reserpine. Apparently, secretion of prolactin of these reserpinized animals was not disturbed since nidation could be stimulated by supplement treatment with daily* 20 μg *FSH. Melatonin and inhibitor of brain monoamine oxidase, Marsilid at* 4 *mg/day and Marplan at* 1 *mg/day, are capable of reversing reserpine effect and induce normal nidation very similar to the effect of FSH. Melatonin could stimulate nidation in* 15 *mg/kg/day reserpinized animals at the dose as small as* 100 μg/*day but other monoamines and precursors are relatively ineffective even at far higher dose.*

Both melatonin at 3×6 μg/*day and its related alkylamine serotonin at* 3×12 μg/*day could improve the incidence of blastocyst implantation in reserpinized animals by direct application into the lateral ventricle of the brain. No such effect is observed with* 3×10 μg/*day of noradrenaline. These data favor the possible participation of the pineal hormone and possibly other related alkylamines including serotogenic neurohumor in central regulation of the release of pituitary FSH component of the luteotrophic complex at least during the progestational stage of pregnancy.*

Introduction

Regulation of corpus luteum function is a complex mechanism and not universal among various groups of placental mammals[1,2]. In the rat, prolactin alone is sufficient to support corpus luteum in hypophysectomized animals[3]. However, prolactin is not the only luteotrophic hormone in other mammalian species[4], since it has been repeatedly demonstated that LH alone, although having luteolytic activity in rats[5,6], is the effective hormone to stimulate prompt progesterone secretion from lutein tissues of most species including rats and hamsters[7,8]. In the rat, prolactin may exert either luteotrophic or luteolytic effect depending on physiological conditions of the corpus luteum[9]. In the golden hamster, it was reported that the combination of prolactin and FSH was required for the maintenance of pregnancy in hypophysectomized animals and these two hormones were named the "luteotrophic complex" in this species[10].

Regarding the central regulation of the gonadal function, it has been well established that pituitary release of FSH and LH is controlled by a nanopeptide neurohormone named FSH and LH-Releasing Hormone[11]. Secretion of this neurohomone is regulated by the synaptic mediators in the hypothalamus [12,13]. The control mechanism of prolactin secretion is presumed to be in the opposite direction to the regulation of FSH and LH secretion[14], and mainly regulated by another neurohormone named Prolactin Inhibiting Factor (PIF)[15]. In animals such as rats which have prolactin dominant during the entire period of corpus luteum life, it has been shown that direct application of dopaminergic and adrenergic neurohumors stimulate secretion of pituitary FSH and LH while alkylamines of serotoninergic neurohumor and the pineal hormone, melatonin, stimulate secretion of prolactin[12]. At the present time, the role of biogenic monoamines on regulation of luteotrophic hormone(s) secretion required for blastocyst implantation has not yet been critically determined. The purpose of this study is to find out if pregnancy in the golden hamster, one of the species closely related with the rat, whose minimal requirements for the maintenance of corpora lutea function are FSH and prolactin, is controlled by any biogenic monamines present in mammalian brain and/or the pineal tissue. Particular interest was stessed on the regulation of the FSH component of the pituitary luteotrophic complex by using the central depletor of monoamines, reserpine[16], one of the tranquilizing drugs which is capable of inhibiting FSH and LH secretion but still actively stimulates prolactin secretion[17,18]. It was also investigated whether any supplementary treatment with individual biogenic monoamines and monoamine oxidase inhibitors could maintain corpus luteum function. Preliminary results were presented in abstracts [19,20].

Materials and Methods

One hundred and seventy virgin adult female hamsters of the Department of Biology colony were used in this study. They were 2–3 months old and showed regularity of 4-day estrous cycle. Proestrous females (i.e. 3 days after the gross appearance of the post-estrous discharge of the viscous fluid in the vaginal lumen[21], Fig. 1) were induced pregnancy by exposing to 3–6 months old males of known fertility. Only animals with sperms or plug present in the vaginal smear were used. For convenience, the day on which sperms were present in the post-estrous discharge fluid is considered as L_0 of pregnancy and the following day as L_1, L_2, L_3, respectively.

Animals were treated daily with varying doses of reserpine (Union Drug Company, Bangkok) ranging from 5.0 to 100.0 mg/kg from L_0 until L_4 of pregnancy. In addition, each group of animals were also injected with either bovine FSH (Mann Resarch Lab.), melatonin (Calbiochem), serotonin (5–HT, Calbiochem), 5-HTP (Sigma), L-dopa (Sigma), dopamine (Sigma), noradrenaline (NA, Sigma), *p*-chlorophenylalanine (Calbiochem), Monoamine Oxidase Inhibitor (MAOI; Marplan and Marsilid, Rocha Hoffman) alone or in combination with reserpine in order to find out if any of these agents could alter reserpine effect by stimulating FSH component of the pituitary luteotrophic complex and induce nidation at normal time. Reserpine was injected subcutaneously around the back region. Other agents were either injected intraperitoneally or directly applied thrice daily into the lateral ventricle of the brain in order to find out if any of these drugs acts centrally or peripherally on regulation of corpus luteal function and nidation (Fig. 2).

Animals were secrificed on L_6 The presence or absence of implantation sites were recorded and aimed to used as biological parameter for continuation of endogenous secretion of progesterone from the corpora lutea[22] due to the availability of prolactin and FSH[10].

Results

Results are presented in Tables I-V. It will be seen in Table I that the minimal effective dose of reserpine to prevent nidation in the hamster is about 10–15 mg/kg/day. It is important to note that the effective dose of reserpine for prevention of nidation in the hamster is pharmacological rather than physiological since all animals showed typical reserpine symptoms including arching of the back, diarrhea and in many cases animals were unable to eat and drink. However, almost all of them survived during the entire period of reserpine injection and responded quite well to exogenous FSH and alkylamines to undergo normal nidation at expected time (Table II).

Table II shows that subsequent treatments with FSH and melatonin were very effective in reversing reserpine effect and inducing nidation at the expected time. The minimal effective dose of systemic melatonin injection was 100 μg/day. Moreover, MAOI (Marplan and Marsilid) at the dose of 1–4 mg/day were also very effective in reversal of reserpine effect and induce nidation in most cases.

Table III shows that a single injection of various monoamines approximately 1 1/2 days prior to the expected nidation time are not sufficient to induce nidation in reserpinized animals, although 100 μg melatonin treatment is still capable of stimulating nidation to 45.4%.

Table IV shows that direct application of alkylamines but not catecholamine (NA) into the ventricle of the brain several times a day improves the incidence of nidation in reserpinized animals even when the dose is as small as 15–36 μg/day.

Table V shows that injection of the specific depleter of brain serotonin *p*-chlorophenylalanine (p-CPA) [23,24] alone is insufficient to inhibit nidation even at a dose as high as 20 mg/day. However daily injection of this drug can act in synergism with small dose of reserpine (5 mg/kg) and prevent nidation in all cases. On the contrary the precursor of catecholamine L-dopa is unable to inhibit nidation even in the presence of 5 mg/kg reserpine.

Discussion

The minimal effective dose of daily reserpine treatment for inhibition of blastocyst implantation of the hamster observed in this study is approximately ten times higher than the rat, the species which require estrogen in addition or progesterone for initiation of nidation[25]. Although animals exhibited serious reserpine symptoms these effects did not interfere with the role of FSH and monoamines injection, maintenance of corpus luteum function and initiation of nidation at least up to the first few days or post-implantation.

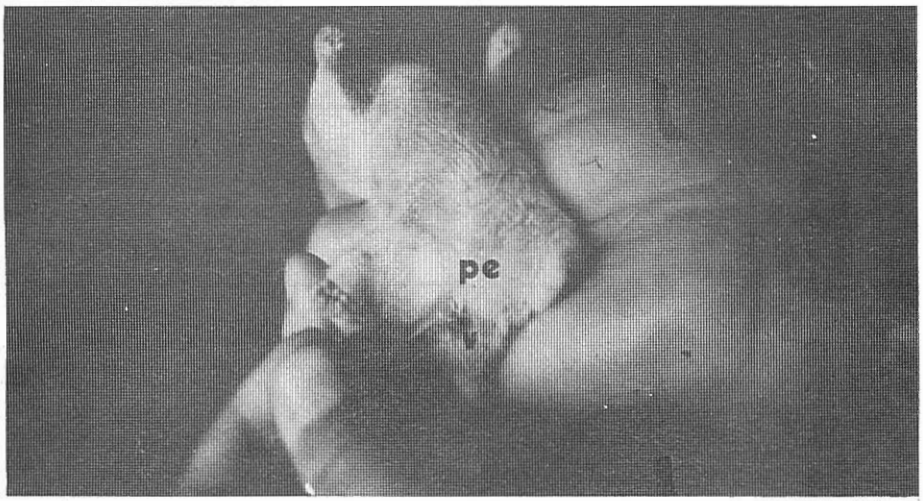

Fig. 1. A typical example of the post-estrous discharge observed in the vaginal lumen of cycling female hamsters every 4 days. Abbreviations: pe=post-estrous discharge, v=vagina

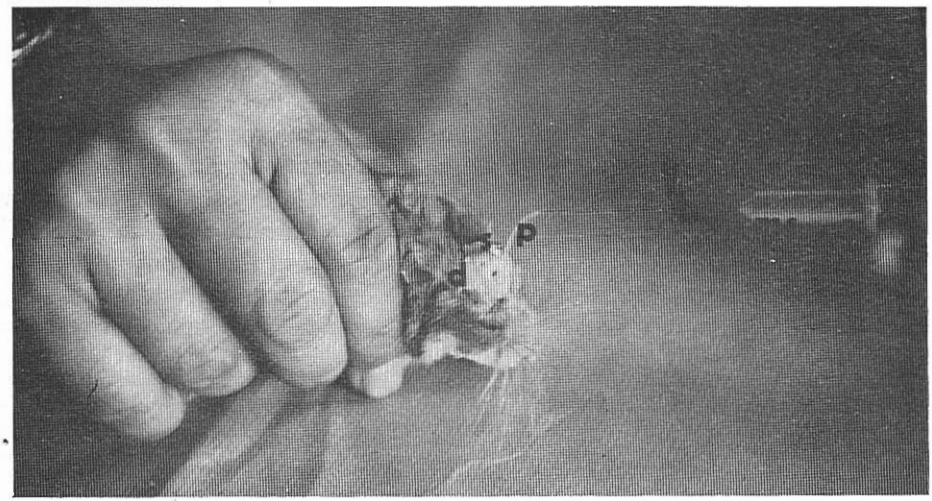

Fig. 2. Illustration of the method of intra-ventricular injections of various biogenic monoamines. The polyethylene tube (Clay Adams) has to be sealed with heat at the end of each injection. Location of the lateral ventricle was achieved by the aid of rodent's stereotaxic apparatus and the hamster's brain atlas[29]. Volume of each injection was arranged not to exceed 10 µl. Abbreviations:
d=dental cement, ms—microsyringe of 25 µl capacity,
p=polyethylene tube with inside diameter 0.030′ and outside diameter 0.048′,
s=screw

Table I

Prevention of nidation in post-coitus pregnant hamsters by reserpine injection.

Dose of reserpine treatment[a] mg/kg/day	Number of animals	L_6 observation			
		Nidation/Total Ratio	%	No. implantation sites/ Each implanted animal[b]	
0.0	29	25/29	86.2	10	(5–13)
5.0	9	9/9	100.0	11	(7–13)
10.0	7	1/7	14.3	10	(10)
15.0	18	1/18	5.6	12	(12)
100.0	4[c]	0/4	0.0		

[a] Reserpine were injected subcutaneously at 1000 hour.

[b] The ranges are indicated in parentheses.

[c] Animals died prior to L6 were excluded.

It is well established that tranquilizers such as reserpine has inhibitory effect on pituitary gonadotrophin secretion while it facilitates secretion of prolactin in most mammalian species including human[17,18,26]. Evidence shown in this study further indicated that it was possible to suppress gonadotrophin release in the species which require FSH in addition to prolactin for maintaining the functional life of the corpus luteum by the tranquilizer without any apparent influence on prolactin secretion because all animals treated with 10–15 mg/kg/day reserpine would nidate at expected time by simultaneous injection with 20 μg FSH only.

Evidences of possible involvement of monoamines on regulation of FSH component of the pituitary luteotrophic complex are overwhelming since inhibitor of brain monoamine oxidase Marplan and Marsilid were very effective in reversal of reserpine effect on inhibition of nidation. Moreover daily injection of individual monoamines showed that melatonin was very effective in inducing nidation in reserpinized animals irrespective of the route of administration but the precursor of melatonin serotoninergic neurohumor[27] was effective only when directly applied into the ventricular system of the brain. Whether both alkylamines are specific regulator of the release of FSH component of the pituitary luteotrophic complex remains to be determined. However it is certain that at least serotonin is not the sole agent for stimulation of pituitary FSH secretion since p-CPA, the specific depleter of brain monoamine alone is unable to prevent nidation in the

Table II

Stimulation of blastocysts implantation in reserpinized post-coitus pregnant hamsters by FSH, monoamines and precursors and Monoamine Oxidase Inhibitor (MAOI).

Reserpine was injected subcutaneously at 10.00 hour, all other substances were injected intraperitoneally one hour prior to reserpine injection.

L_0–L_4 Treatment	Number of animals	L_6 Observation			
		Nidation/Total Ratio	%	No. Implantation Sites/Each Implanted animal[a]	
1. *Reserpine+FSH*					
a. 15 mg/kg+0.02 mg	5	5/5	100.0	12	(9–14)
b. 100 mg/kg+0.02 mg	6	3/6	50.0	11	(11–12)
2. *Reserpine+5-HTP*					
a. 15 mg/kg+0.05–0.1 mg	15	4/15	26.7	8	(3–13)
b. 15 mg/kg+1.0 mg	6	0/6	0.0		
3. *Reserpine+5-HT*					
a. 15 mg/kg+1.0 mg	3	1/3	33.3	12	(12)
b. 15 mg/kg+2.0–4.0 mg	10	4/10	40.0	9	(2–16)
4. *Resespine+Melatonin*					
a. 14 mg/kg+0.01 mg	4	1/4	25.0	10	(10)
b. 15 mg/kg+0.1 mg	3	2/3	66.7	11	(7–14)
c. 15 mg/kg+0.25 mg	5	5/5	100.0	12	(9–15)
5. *Reserpine+L-Dopa*					
a. 10 mg/kg+5.0 mg	6	2/6	33.3	12	(11–12)
b. 15 mg/kg+5.0 mg	14	2/14	14.3	14	(13–15)
6. *Reserpine+MAOI*					
a. 15 mg/kg+Marsilid 4.0 mg	7	7/7	100.0	10	(6–13)
b. 15 mg/kg+Marplan 1.0 mg	9	7/9	77.8	12	(5–17)

[a] The ranges are indicated in parentheses.

Table III

Comparative affect of a single injection of various monoamines on induction of nidation in reserpine treated pregnant hamsters.

All monoamines were injected intraperitoneally one hour prior to reserpine injection. All animals were treated subcutaneously with 15 mg/kg/day at 1000 hour starting from L_0 until L_4 of pregnancy.

Treatment	Number of animals	L_6 Observation			
		Nidation/Total Ratio	%	No. implantation sites/Each implanted Animal[a]	
1. Reserpine control	18	1/18	5.6	12	(12)
2. +5–HTP 100 μg (L_3)	4	0/4	0.0	0	
3. +Melatonin 100 μg(L_3)	11	5/11	45.4	8	(4–11)
4. +Dopamine 100 μg(L_3)	4	0/4	0.0	0	

[a] The ranges are indicated in parenthreses.

Table IV

Comparative effect of L_0-L_4 intra-ventricular (lateral ventricle) application of monoamines on induction of nidation in reserpinized pregnant hamsters.

All monoamines were injected at 700, 1200 and 1700 hour. All animals were treated subcutaneously with 15 mg/kg/day at 1000 hour starting from L_0 until L_4 of pregnancy

Treatment	Number of animals	L_6 Observation			
		Nidation/Total Ratio	%	No. implantation Sites/Each implanted animal[a]	
1. Reserpine+Vehicle (saline) control (3 x 10 μg/day)	3	0/3	0.0		
2. +5–HT (3 x 12 μg/day)	5	4/5	80.0	6	(1–13)
3. +Melatonin (3 x 5 μg/day)	5	4/5	80.0	10	(6–14)
4. +NA (3 x 10 μg/day)	3	1/3	33.3	2	(2)

[a] The ranges are indicated in parentheses.

Table V

Effect of depletor of brain serotonin, *p*-cholorophenylalanine, and precursor of catecholamines, L-dopa, alone or in combination with sub-minimal dose of reserpine on nidation in post-coitus pregnant hamsters.

Number of animals	L_0–L_4 Treatments			L_6 Observation			
	*p*CPA.[a] mg (i.p.)[b]	L–Dopa mg (s.c.)[c]	Reserpine mg/kg (s.c.)[c]	Nidation/Total Ratio	%	No. implantation sites/Each implanted animal[d]	
5	20	—	—	5/5	100.0	12	(10–15)
6	10	—	5.0	0/6	0.0		
6	5	—	5.0	3/6	50.0	12	(9–15)
12	—	0.25–0.5	—	13/13	100.0	11	(9–12)
12	—	1.0–2.0	—	12/12	100.0	10	(3–13)
12	—	5.0–10.0	—	12/12	100.0	11	(9–13)
4	—	5.0	5.0	4/4	100.0	12	(11–14)

[a] *p* Chlorophenylalanine.

[d] Intraperitoneal injection.

[c] Subcubaneone injection.

[b] The ranges are indicated in parentheses.

hamster. Finally, a single injection of melatonin could stimulate nidation in nearly half of reserpinized animals. Furthermore, the recent finding of close anatomical relationship between the hamster pineal gland and its ventricular system of the brain [28] may further strengthen the possibility of physiological involvement of the pineal hormone(s) on regulation of pituitary FSH secretion.

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References

1. Brambell, F.W.R. (1956) in *Marshall's Physiology of Reproduction* (Parkes, A.S., ed.) 3rd Ed., vol. I (I), pp. 397–452, Longman, Green & Co., London.
2. Greenwald, G.S. (1969) *Progess in Endocrinology, Proceedings of the Third International Congress in Endocrinology, Maxico, D.F., June* 30–*July* 5, 1968, pp. 921–926, Excerpta Medica Foundation, ICS 184.

3. Astwood, E.B. (1941) *Endocrinology* **28,** 309–320.
4. Greep, R.O. (1961) in *Sex and Internal Secretion* (Young, W.C. ed.), 3rd Ed., vol. I, pp. 240–304, Williams and Wilkins, Baltimore.
5. Bunde, C.A. and Greep., R.O. (1936) *Proc. Soc. Exp. Biol. (N.Y.)* **35,** 235.
6. Rothchild, I. (1965) *Acta Endocrinol.* **49,** 107–119.
7. Armstrong, D.T., O'Brien, J. and Greep, R.O. (1964) *Endocrinology* **75,** 488.
8. Moudgal, N.R. (1973) *Endocrinology, Proceedings of the Fourth International Congress in Endocrinology, Washington, D.C., June* 18–24, 1972, pp. 920–926, Excerpta Medica Foundation, ICS 273.
9. Malven, P.V. and Sawyer, C.H. (1966) *Endocrinology* **79,** 268–274.
10. Greenwald, G.S. (1967) *Endocrinology* **80,** 118.
11. Schally, A.V., Arimura, A. and Kastin, A.J. (1973) *Endocrinology, Proceedings of the Fourth International Congress of Endocrinology, Washington, D.C., June* 18–24, 1972, pp. 93–97, Excerpta Medica Foundation, ICS 273.
12. Kamberi I.A. (1973) *Endocrinology, Proceedings of the Fornth International Congress of Endocrinology, Washington, D.C., June* 18–24, 1972, pp. 112–119. Excerpta Medica Foundation, ICS 273.
13. Segal, S.J. (1974) *Sci. Am.* **231 (3),** 53–62.
14. Rothchild, I. (1962) *Fertil. Steril.* **13,** 246–258.
15. Meites, J. (1973) in *Methods in Investigative and Diagnostic Endocrinology* (Berson, S.A. and Yalow, R.S. eds.), vol. 2 A, pp. 609–615, North Holland Publishing Company, Amsterdam.
16. Maan, H.H. van and Smelik, P.G. (1968) *Neuroendocrinology* **3,** 177.
17. Psychoyos, A. (1968) *J. Reprord. Fertil, Suppl.* **44,** 47–59.
18. Varavudhi, P. (1969) *J. Endocrinology* **43,** 237–245.
19. Varavudhi, P. and Pinyawat, V. (1971) *Abstracts of Fourth Asia & Oceania Congress of Endocrinology, 31st January to 6th February* 1971 *Auckland, New Zealand,* p. 125.
20. Varavudhi, P. and Chobsieng P. (1973) *IX Acta Endocrinologica Congress, Oslo, 17th–21th June,* 1973. *Acta Endocrinol. Suppl.* **177,** 78.
21. Orsini, M.W. (1961) *Proc. Anim. Care Panel* **14,** 193–206.
22. Orsini, M.W. and Meyer, R.K. (1962). *Proc. Soc. Exp. Biol. Med.,* **110,** 713–715.
23. Koe, B.K., and Weissman, A. (1966) *J. Pharmacol. Exp. Ther.* **153,** 499–516.
24. Boyd, A.E., Lebovitz, H.E. and Pfiffer, J.B. (1970) *New Engl. J. Med.* **283,** 1425.
25. Varavudhi, P. (1965) Ph.D. Thesis, The Weizmann Institute of Science, Israel.
26. Mayer, G. (1965) in *Agents Affecting Fertility* (Austin, C.R. and Perry, J.S. eds.), pp. 291–306 Churchill London.

27. Wurtman, R.J. (1969) *Progress in Endocrinology, Proceedings of the Third International Congress of Endocrinology, Mexico, D.F., June* 30-*July* 5, 1968, pp. 267–230, Excerpta Medica Foundation, ICS 184.

28. Douglas, W.W. (1971) in *The Pharmacological Basis of Therapeutics* (Goodman, L.S. and Gilman, A., eds.) 4th Ed. pp. 645–654.

29. Knigg K.M. and Shirley A.J. (1968) in *The Golden Hamster* (Hoffman, R.A., Robinson, P.F. and Magalhes eds.), pp. 285–319, The Iowa State Univ. Press.

บทคัดย่อ

ยากดประสาทชนิดรีเซอบีน เมื่อฉีดเข้าไปในแฮมสเตอร์ภายหลังจากผสมกับตัวผู้ทุก ๆ วัน ในปริมาณ 10—15 มิลลิกรัม/กก. สามารถมีผลห้ามต่อมใต้สมองส่วนหน้าไม่ให้หลั่ง **FSH** ที่จำเป็นร่วมกับโปรแลกติน สำหรับกระตุ้นให้คอร์ปัส ลูเตียม ทำงานทำให้บลาสโตซีส ไม่สามารถฝังตัวที่ผนังมดลูกได้ แต่ โปรแลคติน ยังคงหลั่งออกม่าเป็นปกติ ทั้งนี้เพราะการฉีด **FSH** ทดแทนเพียง 20 ไมโครกรัม/วัน ก็สามารถกระตุ้นคอร์ปัส ลูเตียม ให้ทำงานและมีบลาสโตซีสฝังตัวที่ผนังมดลูกได้เป็นปกติ เมื่อใช้ฉีดเมลาโตนินและตัวห้ามเอ็นไซม์ โมโนแอมีนอ๊อกซิเดสชนิด **Marplan** 1 มิลลิกรัม/วัน และ **Marsilid** 4 มิลลิกรัม/วัน แทน **FSH** ในสัตว์ทดลองที่ฉีดรีเซอบีน 15 มิลลิกรัม/กก./วัน พบว่าสามารถกระตุ้นคอร์ปัส ลูเตียม ให้ทำงานและมีการฝังตัวของบลาสโตซีสได้เป็นปกติในสัตว์ทดลองเกือบทั้งหมดที่ศึกษา แต่โมโนแอมีนส์ตัวอื่น ๆ ไม่มีผลให้เห็นชัดเจน เมื่อฉีดเข้าไปโดยตรงที่ช่องว่างภายในสมองวันละ 3 ครั้ง พบว่าโมโนแอมีนส์ กลุ่มอัลคีลามีนส์ คือ เมลาโตนิน 3×6 ไมโครกรัม/วัน และ เซอโรโตนิน 3×12 ไมโครกรัม/วัน กระตุ้นคอร์ปัส ลูเตียม ให้ทำงานได้ดี แต่แคทีโคลามีนชนิดนอร์แอดรีนาลีน 3×10 ไมโครกรัม/วัน ไม่มีผล

ผลการทดลองสรุปได้ว่า ฮอร์โมนจากต่อมไพเนียลและกลุ่มอัลคีลามีนส์ตัวอื่น ๆ ซึ่งอาจรวมทั้งฮอร์โมนประสาทชนิดเซอโรโตนินมีบทบาทสำคัญในการควบคุมการหลั่ง **FSH** ซึ่งมีส่วนจำเป็นในการควบคุมการทำงานของคอร์ปัส ลูเตียม ในระยะแรก ๆ ของการตั้งครรภ์