

Effect of Calcium on Estrogen and Follicle Stimulating Hormone Secretion in Rabbits

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Abstract □ The effect of calcium gluconate on estrogen (estradiol) serum level as well as follicle stimulating hormone level was studied. Our results revealed that oral administration of calcium gluconate (100 mg/kg body weight) to adult non-pregnant female rabbits caused a significant increase of serum levels of estradiol and follicle stimulating hormone. On the other hand, oral contraceptive (Norminest tablets) decreased significantly follicle stimulating hormone serum level, while combined administration of calcium gluconate and oral contraceptive caused significant increase of serum level of follicle stimulating hormone compared with control values. Also, concurrent administration of calcium gluconate and Norminest tablet increased significantly the rate of conception compared with group received Norminest tablets only. These results indicated that combined administration of calcium and oral contraceptives must be cautiously.

Keywords □ Calcium gluconate, follicle stimulating hormone, estrogen.

The relationship between calcium and estrogen secretion and/or estrogen metabolism was not intensively investigated until now. There are a few literatures which relate estrogen metabolism to calcium level. Tsang *et al.*¹⁾ stated that feeding of laying hen with calcium-deficient diet reduced the metabolic clearance rate of intravenously injected 3H-estradiol-17 β . This is due to inhibition of major estrogen metabolic pathway, namely sulfation, *i.e.*, calcium deficiency decreased the conversion of estradiol-17 β -3-sulfate to estradiol-17 α -3-sulfate with a concomitant reduction of metabolic clearance rate of estradiol-17 β from plasma.

Boucher *et al.*²⁾ mentioned that estrogen therapy decreased serum total and ionized calcium concentration in postmenopausal women. This decrease was evident at 3 weeks and persisted for 23 weeks. On the other hand Russell *et al.*³⁾ stated that estrogen therapy decreased serum calcium levels in rats due to inhibition of calcium absorption, while Osa and Maruta⁴⁾ study made on uterine longitudinal muscles of spayed and estrogen treated rats showed that estradiol inhibit the cell membrane permeability to divalent cations including calcium. The effect which may suggest that estrogen may increase the serum level of calcium.

Riis *et al.*⁵⁾ and Ettinger *et al.*⁶⁾ suggested that calcium supplementation does not potentiate the effect

of estrogen therapy in prevention of the early postmenopausal bone loss.

In our survey made on the relationship between calcium and estrogen, we did not find any study which investigate the effect of calcium on serum estrogen level. The present work is a trial to clarify the relationship between calcium supplementation and serum estrogen and follicle stimulating hormone (F. S. H.) levels.

MATERIALS AND METHODS

Drugs

Calcium gluconate (Misr).

Oral contraceptive (Norminest Tablets) (Syntex Lab.): each tablet contains, norethindrone 0.5 mg and ethinyl estradiol 0.035 mg.

Animals and mating

The study was performed on twenty-four adult non-pregnant female rabbits weighing 1.75-2.0 kg. For mating, one male rabbit was used for all females. Female rabbits were caught from the fur of the neck region by one hand, and the tail was tied and left up by a thread, and then introduced to the male rabbit. Mating was confirmed by observation of the actual coupling between male and female,

Table I. Effect of calcium gluconate (100 mg/kg) on serum level of estradiol (pg/ml) before and 2 hours after treatment

Treatment	Estradiol (pg/ml)			Mean diff. \pm S.E
	Before	After	% diff.	
-Calcium	16.5	51.8*	214	35.3 \pm 3.8

Results of mean and mean difference \pm standard error of the mean (Mean diff. \pm S.E.).

* $p < 0.01$

Table II. Effect of calcium gluconate (100 mg/kg) and/or oral contraceptives (Norminest tablets) (one tablet/8 ml distilled water & given 1 ml/kg body weight) on serum level of follicle stimulating hormone (F.S.H.) (mL.U./mL) before and 2 hours after treatment

Treatment	F.S.H. (mL U./mL)			Mean diff. \pm S.E.
	Before	After	% diff.	
Calcium	1.5	3.7*	147	2.2 \pm 0.7
Oral contraceptive	4.1	2.3*	44	1.8 \pm 0.6
Calcium + Oral contraceptive	1.2	4.7*	292	3.5 \pm 0.6

Results of mean and mean difference \pm standard error of the mean (Mean diff. \pm S.E.).

* $p < 0.05$

then male was separated.

Drug administration

Animals were divided into 4 groups. Blood samples were collected from the marginal ear vein before and 2 hours after the administration of calcium and/or oral contraceptives. For the first group, a single dose of calcium gluconate (100 mg/kg)⁷ was given by the oral route. Estradiol and F.S.H. were determined. For the second group, one tablet of Norminest was dissolved in 8 ml of distilled water and each animal was given 1 ml/kg, orally⁷. F.S.H. was determined, and the rate of conception was observed. For the third group, calcium gluconate was given in combination with Norminest tablets in the previously mentioned dose levels. F.S.H. was determined, and the rate of conception was also observed. The fourth group was served as control for the determination of the rate of conception with second and third groups.

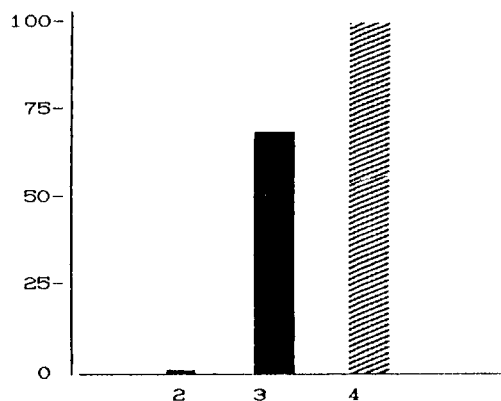


Fig. 1. Rate of conception after administration of oral contraceptive (Norminest tablet) with zero % group 2 and after combined administration of calcium gluconate and oral contraceptive (66.7% conception) group 3 compared with control group (100% conception) group 4.

Serum were separated and used for the determination of estrogen as estradiol and/or F.S.H. Estradiol was determined by the ENDAB unconjugated estradiol enzyme immunoassay kit catalog No. 117. F.S.H. was determined by EUROGENETICS monoclonal antibody-based enzyme immunoassay.

Statistical analysis

Statistical analysis of the results were carried out according to Paired *t*-test⁸. The difference was considered significant if $p < 0.05$.

RESULTS

Oral administration of calcium gluconate in a dose of 100 mg/kg body weight to adult non-pregnant female rabbits produced a significant increase of estradiol and F.S.H. serum levels with percentage increase exceeded 210% & 140% respectively. While administration of oral contraceptive alone (1 ml of the prepared solution of Norminest tablets/kg body weight) caused about 44% decrease of F.S.H. serum level (Table I, II).

Oral administration of combination of calcium gluconate and oral contraceptive (Norminest tablets) caused about 290% increase of F.S.H. serum level (Table II) and at the same time increased the rate of conception (as indicated by increased rate of pregnancy and delivery after the normal conception period 30-31 days) by about 67% (Fig. 1), *i.e.*, concurrent administration of calcium gluconate and oral contraceptives

abolished the inhibitory effect of oral contraceptives on F.S.H.

DISCUSSION

Calcium plays an important role in stimulus-secretion coupling in most exocrine and endocrine glands⁹. Calcium movement into the cell and release from intracellular sites is vital for the coupling of receptor-stimulated cell surface events to cellular responses (*i.e.*, stimulus-response coupling)¹⁰⁻¹². Accordingly we may predict an increase of different hormonal secretions following administration of calcium. The results obtained from the present study indicated that oral administration of calcium gluconate to adult non-pregnant female rabbits caused a significant increase of both serum levels of estradiol and F.S.H.

On the other hand, administration of oral contraceptive together with calcium to female rabbits (in-caged with males) led to higher percentage of conception compared with female rabbits received oral contraceptive only. This may be explained by the fact that calcium maintained a higher serum level of F.S.H. leading to increased rate of ovulation, *i.e.*, calcium antagonized the inhibitory effect of oral contraceptive on F.S.H. A similar results may be obtained in human beings (although it needs more investigations) and accordingly concurrent administration of oral contraceptives and calcium must be avoided.

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