

Ovarian and reproductive tract responses to vitamin and mineral supplementation in prepubertal gilts

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ABSTRACT

The roles of vitamins and minerals in reproductive tract development and attainment of puberty have received very little attention. We examined the effects of vitamin and mineral supplementation on weight and follicular development of ovaries, ovarian concentration of luteinizing hormone (LH) receptors and weight and length of uteri in prepubertal gilts.

Thirty crossbred (Large White x Polish Landrace) 160- (Group I; 75-85 kg) and thirty 180-day-old (Group II; 85-95 kg), randomly assigned gilts received 3100 kcal ME/kg/day (control; n=15) or supplemented (n=15) with 25 g of minerals (MMT-Polfa, Kutno, Poland) for 14 days and were slaughtered immediately thereafter. Additionally, both mineral-supplemented subgroups received a single i.m. injection of vitamins (A, D₃, E, B₁, B₂, B₆, B₁₂, C and PP).

The supplementation treatment did not affect the length of uterine horns or weight of ovaries in either group I or group II. The LH receptor concentration in the ovary (fM/mg protein) was twice as high (P<0.05) in the supplemented than in control gilts in both group I (1.21±0.25 vs 2.52±0.51) and II (1.87±0.23 vs 3.77±0.63).

The total number of follicles was 20 % higher in the ovaries of younger gilts (Group I). In the ovaries of younger gilts supplemented with vitamins and minerals, the number of class 1-3 mm diameter, healthy follicles was significantly higher (P<0.05). The total number of atretic follicles was

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27.5% lower in this subgroup. No significant changes in number or distribution of healthy and atretic follicles were found among the supplemented- and control animals of the older group (Group II).

The results of this study show that supplementation with vitamins and minerals can influence follicular development and stimulate ovarian LH receptor induction in prepuberal gilts.

KEY WORDS: vitamins, minerals, ovary, LH receptors, reproductive tract, gilts

INTRODUCTION

In pigs, as in other species, sexual development is related to developmental changes in the ovaries. Ovarian sensitivity to gonadotropins appears very early, approximately in 8-week-old gilts (Oxender et al., 1979). The effect of gonadotropins on ovarian steroid activity (Elsaesser, 1982; Flowers et al., 1989; Jana et al., 1996) and morphological development of ovaries (Kather and Smidt, 1975; Bolamba et al., 1991; Jana et al., 1997) has been well documented. Among pituitary gonadotropins, the pattern of luteinizing hormone (LH) secretion and its action through LH receptors in the granulosa and theca layers of follicular walls seems to play the most important role in follicular development. The roles of vitamins and minerals in pig reproduction generally, and puberty attainment in particular, have received very little attention (Hughes, 1982). It is very probable that severe restriction of many vitamins and minerals, including vitamin A (Hughes, 1934), vitamin B₁₂ (Johnson et al., 1952) and manganese (Plumlee et al., 1956), may delay sexual development.

Our experiment was, therefore, designed to determine whether vitamin and mineral supplementation can affect weight and follicular development of ovaries, ovarian concentration of LH receptors, weight and length of uteri in prepuberal gilts.

MATERIAL AND METHODS

Thirty 160- (Group I; 75-85 kg body weight - BW) and thirty 180-day-old (Group II; 85-95 kg BW) crossbred (Large White x Polish Landrace) gilts kept on basal diet with sufficient protein, vitamins and minerals (Table 1), were randomly assigned to receive 3100 kcal ME/ kg of feed (2.5 kg) daily (control C; n=15) or supplemented (S; n=15) with 25 g of minerals containing Zn (MMT Polfa, Kutno, Poland) for 14 days. Additionally both supplemented subgroups received a single i.m. injection of vitamins (A - 250 000 IU, D₃ - 125 000 IU, E - 50 mg, B₁ - 12.5 mg, B₂ - 10 mg, B₆ - 6.2 mg, B₁₂ - 15 mg, C - 10 mg and 62.5 mg of thiamin) on day 1 of the experiment. The animals were maintained in individual stalls under natural lighting and temperature conditions. The experiment was performed on one farm in northeastern Poland.

TABLE I

Composition of basal diet*, %

Components	
Soyabean meal (Central Soya: RT-135)	15.0
Wheat	47.5
Barley	37.5
L-lysine	0.30
DL-methionine	0.29
Crude fat	2.20
Ash	4.40
Crude protein	16.2
Crude fibre	3.00

* basal diet provided per kg feed: Ca – 7.8 g; P – 5.6 g; Na – 1.4 g; Fe – 160 mg; Cu – 25 mg; Zn – 70 mg; Mn – 40 mg; J – 0.42 mg; Se – 0.25 mg and vitamins: A – 10000 IU; E – 40 mg; B₁ – 3.75 mg, B₆ – 1 mg and B₁₂ – 30 µg
 content of minerals in MMT per kg: Ca – 170 g; P – 106 g; Na – 38 g; Fe – 3500 mg; Cu – 1000 mg, Zn – 5500 mg, Mg – 3500 mg; J – 20 mg; Co – 20 mg

The gilts were slaughtered on day 15 of the experiment and the reproductive tracts and ovaries were collected and processed. Ovaries collected from each gilt were weighed and then one ovary was immediately frozen in liquid nitrogen for further determination of LH receptors. The other ovary was fixed in Bouin's fluid, embedded in paraffin and serially sectioned. Every 50th section (6 µm) was stained with hematoxylin-eosin. In each section all antral follicles were microscopically counted. All measured follicles were morphologically classified as healthy or atretic, based on such degenerative changes as the presence of pycnotic nuclei and/or local destruction of the basement membrane. The follicles were divided into two size classes: small, <1 mm, and medium, 1.1 to 3 mm in diameter. LH receptor levels were determined as previously described and validated (Zięcik et al., 1989; Jana et al., 1996).

Briefly, ovaries were thawed, homogenized in six volumes (v/w) of 25 mmol/l Tris HCl buffer pH 7.4 containing 2.50 mmol/l of sucrose at 4°C with an Ultra-Torax homogenizer. The homogenate was then filtered through four layers of cheese-cloth, and the filtrate centrifuged for 20 min at 800 g at 4°C. The resulting supernatant was centrifuged further for 1 h at 25000 x g at 4°C and the sediment suspended in 3 ml of ice-cold 25 mmol/l Tris-HCL buffer, pH 7.2, containing 0.1% BSA and 5 mmol/l MgCl₂.

hCG (CR-127) was labelled using the chloramine-T method. Nonspecific binding was measured by the addition of 1 ng unlabelled hCG and was less than 2% of the total [¹²⁵I]-labelled hCG added. The concentrations of LH/hCG, unoccupied binding sites, and association constants (K_a) for hormone binding to receptors were determined using the EBDA computer program (Elsevier BIO-SOFT, Cam-

bridge, UK). Seven subsaturating quantities of unlabelled hCG (0.15-5 ng) were used for each receptor preparation as determined according to the method of Lowry et al. (1951).

Data and statistical analysis

All data were presented as mean \pm SEM and compared by analysis of variance (ANOVA) to establish the overall effect of treatment. In addition, comparisons of means were performed by Duncan's multiple range test or the Bonferroni test.

All calculations were performed using the statistical package GraphPad PRISM (GraphPad Software, San Diego, CA, USA).

RESULTS

Vitamin injection and mineral supplementation did not affect the weight or length of uterine horns of either 160- or 180-day-old gilts (Figures 1 a, c). There was also no difference in the weight of ovaries (Figure 1b) between control and vitamin- and mineral-supplemented gilts. The LH receptor concentration (fM/mg protein) was two times higher ($P < 0.05$) in vitamin- and mineral-treated gilts than controls in both group I (control - 1.21 ± 0.25 vs. treated - 2.52 ± 0.51) and II (control - 1.87 ± 0.23 vs. treated - 3.77 ± 0.63). The association constant (K_a) did not differ among groups and ranged between 1.8 - 2.8×10^{10} l/mol.

The total number of follicles was approximately 20% higher in the ovaries of the younger (Group I) than older (Group II) gilts in controls as well as in the vitamin- and mineral-treated subgroups. In the ovaries of the treated gilts (Group I, Subgroup S) the number of healthy follicles was significantly higher ($P < 0.05$) in class of 1-3 mm diameter than in controls (Table 2). The total number of atretic follicles decreased in this subgroup by 27.5% and healthy/atretic follicles ratios increased from 1.4:1 in controls to 2.0:1 in vitamin- and mineral- treated gilts. No significant change in number and distribution of healthy and atretic follicles was found among gilts in Group II given vitamins and supplemented with minerals when compared with controls (Table 2) and the healthy/atretic follicle ratios remained unchanged (2.0:1 in controls vs 2.1:1 in treated gilts).

DISCUSSION

In the conducted trial, the diet of gilts, prior to and during the experiments, was supplied with vitamins and minerals according to standard nutritional requirements. The excess of vitamins and minerals was applied as „flushing” to alter the repro-

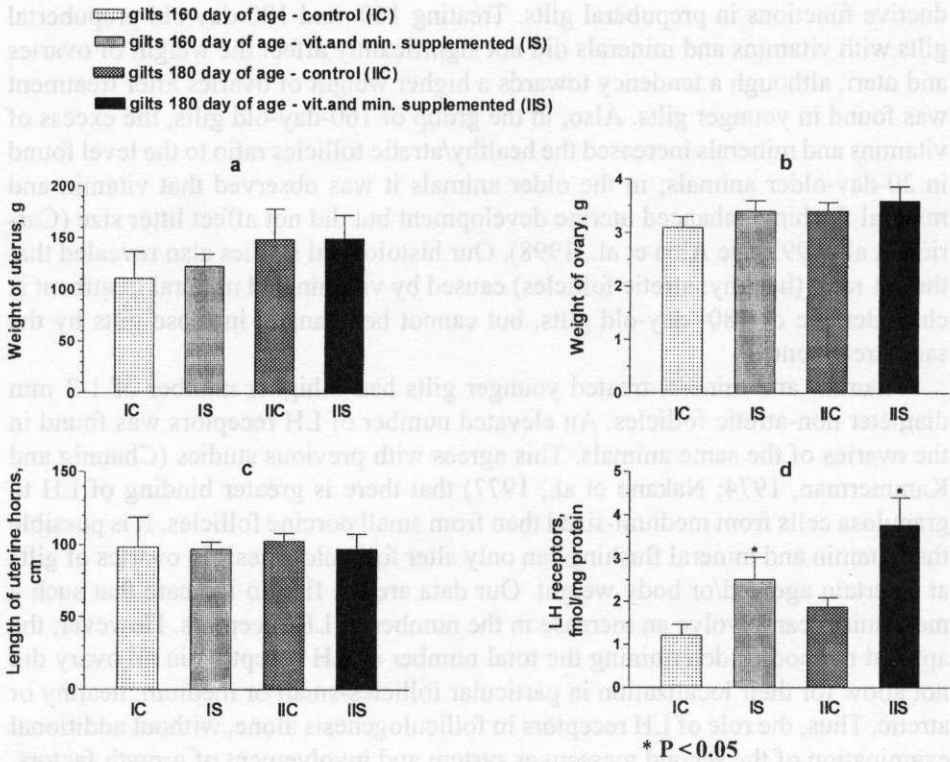


Figure 1. Effects of vitamins and minerals supplementation on weight of ovary (a), weight of uterus (b), length of uterine horns (c) and ovarian LH receptors (d) in 160 (groups IC and IS) and 180 (groups IIC and IIS) days old gilts (mean \pm SEM)

TABLE 2

Number of healthy and atretic follicles in ovaries of 160 and 180 days old gilts supplemented (groups IS and IIS) or not (groups IC and IIC) with vitamins and minerals (every fiftieth 6 μ m section of one ovary)

Age of gilts days	Group n=15	Number of follicles on histological sections			
		healthy		atretic	
		<1 mm	1-3 mm	<1 mm	1-3 mm
160	Control (IC)	244.4 \pm 33.8	29.2 \pm 3.2 ^a	180.5 \pm 19.9	19.6 \pm 2.9
	Vitamins and minerals supplemented (IS)	278.0 \pm 42.6	42.0 \pm 3.8 ^b	134.5 \pm 25.2	22.1 \pm 3.9
180	Control (IIC)	186.9 \pm 22.9	49.5 \pm 5.6	92.4 \pm 18.6	20.0 \pm 2.6
	Vitamins and minerals supplemented (IIS)	224.2 \pm 39.3	47.9 \pm 6.8	93.1 \pm 18.9	23.9 \pm 5.9

^{a, b} P<0.05

ductive functions in prepubertal gilts. Treating 160- and 180-day old prepubertal gilts with vitamins and minerals did not significantly affect the weight of ovaries and uteri, although a tendency towards a higher weight of ovaries after treatment was found in younger gilts. Also, in the group of 160-day-old gilts, the excess of vitamins and minerals increased the healthy/atretic follicles ratio to the level found in 20-day-older animals; in the older animals it was observed that vitamin and mineral flushing enhanced uterine development but did not affect litter size (Carrión et al., 1993; De Alba et al., 1998). Our histological studies also revealed that the 2:1 ratio (healthy: atretic follicles) caused by vitamin and mineral treatment is characteristic of 180-day-old gilts, but cannot be changed in those gilts by the same treatment.

Vitamin- and mineral-treated younger gilts had a higher number of 1-3 mm diameter non-atretic follicles. An elevated number of LH receptors was found in the ovaries of the same animals. This agrees with previous studies (Channig and Kammerman, 1974; Nakano et al., 1977) that there is greater binding of LH to granulosa cells from medium-sized than from small porcine follicles. It is possible that vitamin and mineral flushing can only alter folliculogenesis in ovaries of gilts at a certain age and/or body weight. Our data are the first to indicate that such a mechanism can involve an increase in the number of LH receptors. However, the applied method of determining the total number of LH receptors in an ovary did not allow for their localization in particular follicles-small or medium, healthy or atretic. Thus, the role of LH receptors in folliculogenesis alone, without additional examination of the second messenger system and involvement of growth factors, cannot be overestimated. On the other hand, the elevated number of LH receptors in the ovaries of vitamin- and mineral-treated prepubertal gilts suggests that vitamin and mineral treatment can increase the receptivity of ovaries to gonadotropins.

Nothing is known about toxic levels of added vitamins/minerals. In our studies, negative effects on the reproductive tract and ovarian development were not observed.

CONCLUSIONS

Supplementation of a basal diet with vitamins and minerals did not influence the weight of the reproductive tract in prepubertal gilts. The addition of vitamins and minerals can, however, affect follicular development at a certain stage of prepubertal development in gilts.

REFERENCES

- Bolamba D., Matton P., Sirard M.A., 1991. Ovarian morphological conditions and the effect of injection of human chorionic gonadotropin on ovulation rates in prepubertal gilts with two morphologically different ovarian types. *J. Anim. Sci.* 69, 3774-3779
- Carrion D., Ewan R., 1993. Kinetics of the alcohol and acetate forms of vitamin E after i.m. and i.v. administration to gilts. *Swine Report*. Iowa State University, Iowa, pp. 28-30
- Channing C.P., Kammerman S., 1973. Characteristics of gonadotropin receptors of porcine granulosa cell during follicle maturation. *Endocrinology* 92, 531-540
- De Alba C., Fuentes A., Rioperez J., Zięcik A., García C., Martín Rillo S., 1998. Effect of vitamin and mineral flushing (Vit. A, D3, E and Se) on the development of the genital tract, ovary activity and litter size in gilts. *Proceedings of 15th IPVS Congress*, Birmingham (England), p. 42
- Elsaesser F., 1982. Endocrine control of sexual maturation in the female pig and sexual differentiation of the stimulatory oestrogen feedback mechanism. In: D.J.A. Cole, G.R. Foxcroft (Editors). *Control of Pig Reproduction*. Butterworths, London, pp. 93-116
- Flowers B., Martin M.J., Cantley T.C., 1989. The effect of pregnant mare serum gonadotropin on follicle stimulating hormone and estradiol secretion in the prepubertal gilt. *Anim. Reprod. Sci.* 21, 93-100
- Hughes E.H., 1934. Some effects of vitamin A-deficient diets on reproduction in sows. *Agr. Res.* 49, 943-953
- Hughes P.E., 1982. Factors affecting the natural attainment of puberty in the gilt. In: D.J.A. Cole, G.R. Foxcroft (Editors). *Control of Pig Reproduction*. Butterworths, London, pp. 117-138
- Jana B., Dusza L., Sobczak J., 1996. Effect of exogenous porcine prolactin (pPRL) on LH plasma concentration and on the release of GnRH in vitro from stalk median eminence in prepubertal gilts. *Anim. Reprod. Sci.* 41, 295-304
- Jana B., Dusza L., Sobczak J., Doboszynska T., 1997. Effect of exogenous porcine prolactin on plasma steroid hormones and ovarian morphology in prepubertal gilts. *Endocr. Regul.* 31, 13-21
- Johnsen H.H.K., Moustgaard J., Hojgaard-Olsen N., 1952. The significance of vitamin B12 for the fertility of sows and gilts. *Anim. Breed. Abstr.* 23, 784
- Kather L., Smidt D., 1975. Vergleichende Untersuchungen zur ovariellen Reaktion infatiler weiblicher Schweine der Deutschen Landrasse des Göttinger Miniaturescheines auf gonadotrope Stimulierung. *Zuchthygiene* 10, 10-15
- Lowry O.H., Rosebrough N.J., Faar A.L., Randall R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275
- Nakano R., Akahori T., Katayama K., Tojo S., 1977. Binding of LH and FSH to porcine granulosa cells during follicular maturation. *J. Reprod. Fertil.* 51, 23-27
- Oxender W.D., Colenbrander B., van de Wiel D.F.M., Wensing C.J.G., 1979. Ovarian development in fetal and prepubertal pigs. *Biol. Reprod.* 21, 715-721
- Plumlee M.D., Thrasher D.M., Beeson W.W., Andrews F.N., Parker H.E., 1956. The effects of manganese deficiency upon the growth development and reproduction of swine. *J. Anim. Sci.* 15, 352-367
- Zięcik A.J., Esbenshade K.L., Britt J.H., 1989. Effects of a gonadotrophin-releasing hormone antagonist on gonadotrophin secretion and gonadal development in neonatal pigs. *J. Reprod. Fertil.* 87, 281-289

STRESZCZENIE

Wpływ witamin i dodatków mineralnych na rozwój układu rozrodczego u niedojrzałych płciowo loszek

Znaczenie witamin i składników mineralnych na rozwój układu rozrodczego i osiągnięcie dojrzałości płciowej zwierząt nie było do tej pory przedmiotem większego zainteresowania. W niniejszej pracy badano wpływ witamin i dodatków mineralnych na ciężar i rozwój jajników, zawartość jajnikowych receptorów hormonu luteinizującego (LH) oraz ciężar i długość macicy u niedojrzałych płciowo loszek.

Trzydzieści loszek mieszańców (Wielka Biała x Polska Biała Zwisłoucha) w wieku 160 (Grupa I; 75-85 kg) i trzydzieści w wieku 180 dni (Grupa II; 85-95 kg) dni podzielonych losowo, otrzymywało standardową paszę o zawartości 3100 Kcal/dzien/kg (kontrola n=15) lub z dodatkiem 25 g mieszanki mineralnej (MMT-Polfa Kutno; n=15), przez 14 dni przed ubojem. Dodatkowo zwierzęta z grupy otrzymującej dodatek mieszanki mineralnej otrzymywały domięśniowo iniekcje witamin (A, D₃, E, B₁, B₂, B₆, B₁₂, C i PP).

Witaminy i składniki mineralne nie wpłynęły na długość rogów macicy i ciężar jajników w grupach I i II. Zawartość receptorów LH w jajniku (fM/mg białka) była dwukrotnie większa ($P < 0,05$) u loszek otrzymujących dodatki niż w obu grupach kontrolnych – I ($1,21 \pm 0,25$ vs $2,52 \pm 0,51$) i II ($1,87 \pm 0,23$ vs $3,77 \pm 0,63$). Ogólna liczba pęcherzyków była o 20% większa w jajnikach młodszych loszek (Grupa I). W jajnikach tych loszek otrzymujących dodatki mineralne i witaminy liczba zdrowych pęcherzyków była wysoce istotnie większa ($P < 0,5$) w klasie 1-3 mm średnicy. Jednocześnie liczba pęcherzyków atretycznych była mniejsza w tej grupie o 27,5%. Nie stwierdzono istotnych zmian liczby i rozmieszczenia pęcherzyków zdrowych i atretycznych u loszek otrzymujących dodatki i w grupie kontrolnej u loszek starszych (Grupa II).

Wyniki tych badań wskazują, że witaminy i dodatki mineralne mogą wpływać na rozwój pęcherzyków i stymulować indukcję receptorów LH u niedojrzałych płciowo loszek.