Is 1,25-Dihydroxyvitamin D Required for Reproduction? (42913)

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Abstract. **The role of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) in avian and mammalian reproduction is examined. 1,25-Dihydroxyvitamin D is required, in both the avian and mammalian species, for maintenance of normocalcemia, adequate intestinal calcium absorption, bone turnover, and mineral homeostasis throughout the reproductive cycle—just as it is required in the nonlaying bird or nonpregnant, nonlactating mammal. In the avian species, 1,25-$(OH)_2$D is required for ovulation and shell formation, transfer of calcium from the egg shell across the chorioallantoic membrane to the fetal circulation, and maintenance of fetal serum calcium, bone metabolism, and mineral homeostasis. In the mammalian species, 1,25-$(OH)_2$D is required for normal ovulation, normal fetal and neonatal bone metabolism, milk production, and maintenance of normocalcemia and mineral homeostasis in the neonate. In the absence of 1,25-$(OH)_2$D, however, embryogenesis (rat and chick) and neonatal development (rat) can proceed in such a way as to produce viable, normal appearing offspring. The classical effects of 1,25-$(OH)_2$D deficiency (hypocacemia, inadequate intestinal calcium absorption, and bone mineralization) become increasingly apparent with advancing age but their are no other apparent major developmental abnormalities.** [P.S.E.B.M. 1989, Vol 191]

The importance of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) in mineral metabolism is undisputed. 1,25-Dihydroxyvitamin D stimulates calcium absorption in the intestine and influences both bone mineralization and resorption. Specific cytosolic receptors for 1,25-$(OH)_2$D have been found in intestinal absorptive cells and bone cells, confirming the target nature of these tissues. There is growing evidence, however, that 1,25-$(OH)_2$D may be important in the functioning of other tissues. Receptors for 1,25-$(OH)_2$D have been found in kidney, skin, parathyroid gland, pancreas, thymus, lung, skeletal muscle, pituitary, leukocytes, monocytes, lymphocytes, and of particular relevance to reproduction, in the testes, ovary, avian shell gland, uterus, yolk sac, placenta, and mammary gland (1). Furthermore, 1,25-$(OH)_2$D has been shown to influence cellular differentiation and metabolic activity in many of these tissues and cells. These observations suggest that the function of 1,25-$(OH)_2$D goes well beyond the stimulation of calcium absorption in the intestine and regulation of bone metabolism.

The importance of 1,25-$(OH)_2$D in mineral metabolism and the overwhelming evidence that 1,25-$(OH)_2$D is involved in cellular differentiation and the regulation of metabolic activity in numerous tissues raises the question as to the role of 1,25-$(OH)_2$D in the reproductive cycle. Is 1,25-$(OH)_2$D required for reproduction, and if so, where?

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Aves

Spermatogenesis. Very little is known about the role of 1,25-$(OH)_2$D in spermatogenesis. In early studies, Turk (2) observed that vitamin D-deficient roosters had larger testes, but based on artificial insemination and natural mating studies there was no difference in reproductive efficiency. However, the serum concentration of 1,25-$(OH)_2$D was not measured in these studies and it is not clear, therefore, whether these animals were truly 1,25-$(OH)_2$D deficient. It is not known, therefore, whether proven 1,25-$(OH)_2$D-deficient males can produce viable sperm, and if so whether the number or motility are reduced, or whether sperm from 1,25-$(OH)_2$D-deficient males are capable of fertilization and production of normal offspring.

Ovulation and Egg Shell Formation. Receptors for 1,25-$(OH)_2$D have been found in the chick ovary (3) and shell gland (4, 5), suggesting that 1,25-$(OH)_2$D may be important in ovulation and egg shell formation. In normal vitamin D-replete birds, renal 25-hydroxyvitamin D-1-hydroxylase activity and the serum con-

centration of 1,25-$(OH)_2D$ increase during egg shell formation paralleling the deposition of calcium in the egg shell (6–8). Although not necessary for ovulation to occur (9), the rise in serum 1,25-$(OH)_2D$ stimulates intestinal calcium absorption and may function in the formation and resorption of medullary bone to meet the calcium demands of egg shell formation (7, 10, 11).

If birds are placed on a vitamin D-deficient diet, egg production is dramatically reduced or ceases altogether (12–15). Placing birds on a low calcium diet also stops egg production (15). Since 1,25-$(OH)_2D$ deficiency produces hypocalcemia, it is possible that the decrease in egg production brought about by vitamin D deficiency is a consequence of the decrease in serum calcium and not the deficiency of 1,25-$(OH)_2D$ per se. Furthermore, since birds fed a low calcium diet have high serum 1,25-$(OH)_2D$ concentrations, calcium seems to be a more important determinant of egg production than 1,25-$(OH)_2D$. It is not clear, therefore, whether 1,25-$(OH)_2D$ plays a direct role in ovulation and egg shell formation or simply supports these functions indirectly by maintaining normocalcemia.

Embryogenesis.. Although birds on a vitamin D-deficient diet cease producing eggs, it is possible to restore egg production to normal or near normal by administration of 1,25-$(OH)_2D$ to the hen (13). Administration of 1,25-$(OH)_2D$ restores serum calcium in the hen to normal and also restores egg shell calcium to normal or near normal (16), but hatchability of eggs produced from hens given 1,25-$(OH)_2D$ as their sole source of vitamin D is severely reduced (13). Twenty-day-old embryos from vitamin D-deficient hens given 1,25-$(OH)_2D$ appear normal but are hypocalcemic (16), have poorly mineralized skeletons (16), and mandible development is impaired (13) (Table I). Nevertheless, differentiation and growth of the embryo proceed so as to produce a fully developed, normally appearing chick. The primary problem appears to be a lack of calcium. The embryo is unable to mobilize calcium from the egg shell to support serum calcium and bone mineralization.

To determine whether the hypocalcemia and the skeletal defect in embryos from hens fed 1,25-$(OH)_2D$ arises from an absence of 1,25-$(OH)_2D$ in the embryo, Hart *et al.* (17) studied the transfer of 25-hydroxyvitamin D (25-OH-D) and 1,25-$(OH)_2D$ from hen to egg. Plasma concentrations of 25-OH-D and 1,25-$(OH)_2D$ in 18-day embryos from hens fed 25-OH-D were 1.75 ± 0.19 ng/ml and 177 ± 17 pg/ml, respectively, but in embryos from hens fed 1,25-$(OH)_2D$ as their only source of vitamin D were nondetectable and 4 ± 1 pg/ml, respectively (17). In effect, 18-day-old embryos from hens fed 1,25-$(OH)_2D$ are essentially vitamin D-deficient and presumably this deficiency accounts for the hypocalcemia and deficient skeletal mineralization which in turn impair hatching. Direct administration of 1,25-$(OH)_2D$ to embryos from hens maintained on 1,25-$(OH)_2D$ as their sole source of vitamin D restores serum calcium and phosphorus (18) and increases hatchability (13, 19). These data suggest that 1,25-$(OH)_2D$ is essential for embryonic development in the chicken. The major function of 1,25-$(OH)_2D$ appears to be the stimulation of calcium transport from the shell to embryonic blood through the chorioallantoic membrane. Receptors for 1,25-$(OH)_2D$ have been found in the chorioallantoic membrane (20, 21) and their appearance parallels the transfer of calcium from the shell to the embryonic skeleton (22). 1,25-Dihydroxyvitamin D may also be required for transfer of calcium from the yolk sac earlier in development (23). Although receptors for 1,25-$(OH)_2D$ have been found in a number of other embryonic tissues including intestine, bone, and kidney (22, 24), whether 1,25-$(OH)_2D$ is required for development of these tissues or for any other aspect of embryonic development in the bird remains to be established.

Table I. Egg Shell Calcium, Serum Calcium and Phosphorus, and Tibial Ash Weight in 20-Day-Old Embryos from Hens Maintained on 25-OH-D or 1,25-$(OH)_2D$ as Their Sole Source of Vitamin D(15)

	25-OH-D (2 μg/bird/day)	1,25-$(OH)_2D$ (0.4 μg/bird/day)
Shell calcium (g)	1.68 ± 0.07[a]	1.57 ± 0.12
Plasma calcium (mg/dl)	10.6 ± 0.1	4.2 ± 0.2
Plasma phosphorus (mg/dl)	4.9 ± 0.2	21.4 ± 3.0
Tibial ash weight (mg)	14.7 ± 0.1	6.8 ± 0.3
Tibial % ash	31.2 ± 0.5	24.0 ± 0.9

[a] Mean ± SE.

Mammalia

Spermatogenesis. Receptors for 1,25-$(OH)_2D$ have been found in the seminiferous tubules of rat testes but not in the prostate and only in very low levels in the epididymus (25). Receptor concentrations are low in prepubertal rat testes and increase to mature levels at the time of puberty (25). As in the aves, however, the role of 1,25-$(OH)_2D$ in spermatogenesis remains to be determined.

Ovulation. 1,25-Dihydroxyvitamin D has been reported to increase approximately 2-fold just prior to ovulation (Day 15) (26, 27). Whether this increase has any effect on ovarian function, however, is not known. 1,25-$(OH)_2D$ receptors have been found in ovarian cells and 1,25-$(OH)_2D$ at physiologic concentrations can inhibit the growth of Chinese hamster ovary cells by as much as 60% (3). Despite these observations which clearly suggest that 1,25-$(OH)_2D$ may be important in ovulation, rats maintained on a strict vitamin D-deficient diet from weaning and confirmed 1,25-$(OH)_2D$ deficient by direct measurement of serum 1,25-$(OH)_2D$ levels (i.e., <5 pg/ml) can become pregnant when

mated to vitamin D-replete males and give birth to live offspring (28, 29). Fertility, as judged by the number of females becoming pregnant and giving birth to a healthy litter divided by the total number of days mated, is reduced by 75% and litter size is reduced by 25%. Nevertheless, proven 1,25-$(OH)_2$D-deficient rats can ovulate, become pregnant, and give birth. Whether the decrease in fertility and litter size is a consequence of the absence of 1,25-$(OH)_2$D per se or the hypocalcemia induced by 1,25-$(OH)_2$D deficiency remains to be determined.

Embryogenesis/Maternal Mineral Homeostasis during Pregnancy. Serum 1,25-$(OH)_2$D increases during pregnancy in humans and animals (30–35). The free concentration of 1,25-$(OH)_2$D also increases, at least in the human (Table II) (34). Fetal serum 1,25-$(OH)_2$D concentrations are typically lower than maternal and do not correlate with maternal levels, suggesting that regulation of 1,25-$(OH)_2$D in maternal and fetal circulations occurs independently (31, 33, 35). The rise in maternal serum 1,25-$(OH)_2$D during pregnancy increases intestinal calcium absorption, thereby maintaining normocalcemia and adequate calcium for mineralization of the fetal skeleton (36, 37). In the absence of 1,25-$(OH)_2$D (at least in the rat), intestinal calcium absorption still increases, but the increase is not adequate to maintain normocalcemia (37). These data indicate that, in the rat and under conditions of normal dietary calcium and phosphorus, 1,25-$(OH)_2$D is necessary to maintain maternal mineral homeostasis, just as it is necessary to maintain mineral homeostasis in the nonpregnant animal.

On the contrary, fetal development in the rat is relatively insensitive to the absence of 1,25-$(OH)_2$D. In the absence of detectable 1,25-$(OH)_2$D in maternal serum, body weight, total body calcium, and plasma calcium in 20-day-old fetuses from vitamin D-deficient mothers are normal (Table III) (38, 39). Furthermore, histologic evidence indicates that although osteoid is slightly elevated, longitudinal bone growth and percentage of metaphyseal mineralized tissue are also normal in vitamin D-deficient fetuses (40). In effect, fetal development in the rat (but not necessarily in all species) in the absence of 1,25-$(OH)_2$D is nearly normal. Differentiation, growth, and mineralization of the fetus proceed so as to produce what appear to be normal offspring at the time of parturition with only slight abnormalities in the bone. These data suggest that 1,25-$(OH)_2$D is not required for placental transport of calcium in the rat. Nevertheless, the rat placenta, which can make 1,25-$(OH)_2$D (41), has receptors for the hormone (42) as well as a calcium-binding protein similiar if not identical to the vitamin D-dependent calcium-binding protein found in the intestine (43). Furthermore, 1,25-$(OH)_2$D deficiency has been reported to disrupt fetal calcium homeostasis in sheep, suggesting that placental calcium transport may require 1,25-$(OH)_2$D in some species (44).

Table II. Serum Concentrations of Total and Free 1,25-$(OH)_2$D in Nonpregnant and Pregnant Women (34)

	Nonpregnant	Pregnant
Total 1,25-$(OH)_2$D (pg/ml)	42 ± 3[a]	82 ± 5
% free 1,25-$(OH)_2$D	0.43 ± 0.02	0.36 ± 0.02
Free 1,25-$(OH)_2$D (fg/ml)	177 ± 12	294 ± 24

[a] Mean ± SE.

Table III. Body Weight, Total Body Calcium, and Serum Calcium of 20-Day-Old Rat Fetuses from Vitamin D-Replete and Deficient Mothers (38, 39)

Diet	Litter size	Weight (g)	Total body calcium (mg)	Serum calcium (mg/dl)
+D	12	3.7 ± 0.1[a]	6.4 ± 0.2	11.1 ± 0.1
−D	8	3.8 ± 0.1	7.3 ± 0.4	10.7 ± 0.2

[a] Mean ± SE.

Lactation. In rats, but not necessarily in humans, serum 1,25-$(OH)_2$D is elevated during lactation (30). The rise in serum 1,25-$(OH)_2$D increases intestinal calcium absorption and may function to stimulate bone turnover, making bone mineral available for milk production (37). In the absence of 1,25-$(OH)_2$D, intestinal calcium absorption and bone mineral mobilization still increase but the increases are not adequate to maintain normocalcemia (37). Continuous administration of 1,25-$(OH)_2$D to vitamin D-deficient lactating females, using Alza osmotic minipumps, is fully capable of maintaining serum calcium and phosphorus at or above normal levels (45). These data indicate that 1,25-$(OH)_2$D is required for maintenance of normocalcemia and mineral homeostasis during lactation just as it is during pregnancy and in the nonlactating, nonpregnant animal.

Receptors for 1,25-$(OH)_2$D have been found in the mammary gland (46) and autoradiographic data indicate that 1,25-$(OH)_2$D localizes in alveolar and ductal cells as well as cells of the epidermis of the nipple (47). Rat pups from vitamin D-deficient mothers grow more slowly than pups from vitamin D-replete mothers (38), suggesting a possible defect in mammary gland function as a result of 1,25-$(OH)_2$D deficiency. Analysis of milk from vitamin D-replete and deficient mothers indicate that vitamin D-deficient milk contains elevated levels of fat and the skim fraction contains more protein, calcium, and phosphorus but less carbohydrate than normal milk (48). In contrast, Bhattacharjee *et al.* (49) report a decrease in milk protein content in milk from vitamin D-deficient rats and mice. To determine the nutritional quality of milk from vitamin D-deficient

mothers, Brommage and DeLuca (48) reduced litter size and demonstrated that when vitamin D-deficient mothers were given only two pups to nurse instead of the usual eight, growth was equivalent to that of vitamin D-replete pups. Furthermore, femur dry weight and mineralization were also normal, suggesting that milk from vitamin D-deficient females is nutritionally adequate. In further studies it was demonstrated that vitamin D-deficient females produce insufficient milk to support normal pup growth (48, 50). 1,25-Dihydroxyvitamin D deficiency, therefore, can change both quantity and quality of milk produced. Increasing dietary calcium in vitamin D-deficient females increases milk production as judged by pup growth, suggesting that the hypocalcemia associated with 1,25-$(OH)_2D$ deficiency may be responsible, at least in part, for the fall in milk production (51). In *in vitro* studies, the synthesis of milk proteins in mammary gland explants from vitamin D-deficient animals is reduced (49). Addition of 1,25-$(OH)_2D$ to the culture medium does not restore synthesis, but pretreatment for 10 days with 1,25-$(OH)_2D$ *in vivo* can, suggesting that 1,25-$(OH)_2D$ may be acting indirectly. Based on these data, it is not clear whether 1,25-$(OH)_2D$ plays a direct role in mammary gland function or simply supports normal function by maintaining normocalcemia.

Neonatal Development. Growth of pups born to vitamin D-deficient mothers is impaired. Direct administration of vitamin D to pups nursing a vitamin D-deficient mother, however, has no effect on growth (50). Combined with what we know about milk production in vitamin D-deficient mothers, these data suggest that the growth defect is a consequence of inadequate milk production by the mother and not a deficiency of 1,25-$(OH)_2D$ per se. When adequate milk is provided, growth and skeletal development of vitamin D-deficient rat pups up to 20 days of age is essentially normal, with the exception of an increase in cortical porosity (Table IV) (52). As the pups age, however, receptors for 1,25-$(OH)_2D$ appear in the intestine and the classical effects of vitamin D deficiency become increasingly apparent. It appears that during early neonatal development the animal is capable of maintaining near normal mineral homeostasis and growth in the absence of 1,25-$(OH)_2D$ but as the animal ages the need for 1,25-$(OH)_2D$ increases. Although 1,25-$(OH)_2D$ is clearly required for maintenance of calcium homeostasis after weaning, proven 1,25-$(OH)_2D$-deficient offspring from vitamin D-deficient mothers (i.e., serum 1,25-$(OH)_2D$ concentrations less than 5 pg/ml) continue to grow and thrive for at least 6 months postpartum. Taken collectively, these data indicate that rats can reproduce in the absence of 1,25-$(OH)_2D$, but that fertility is reduced and mineral homeostasis is disrupted in both mother and offspring. Whether other species can reproduce without 1,25-$(OH)_2D$ remains to be determined.

Table IV. Effect of Litter Size on Skeletal Status of Rat Pups 20 Days Postpartum Born to Vitamin D-Replete and Deficient Mothers (52)

Diet	Litter size	Femur length (mm)	Femur ash (mg)	Metaphysial osteoid volume (%)	Cortical porosity (%)
−D	2	17 ± 1[a]	49 ± 2	2.6 ± 0.1	40 ± 1
+D	12	17 ± 1	43 ± 2	1.7 ± 0.2	29 ± 1
+D	8	17 ± 1	56 ± 3	2.7 ± 0.2	23 ± 1
−D	8	15 ± 1	29 ± 1	6.5 ± 0.4	37 ± 1

[a] Mean ± SE.

Summary

It is clear that 1,25-$(OH)_2D$ is required, in both the avian and mammalian species, for maintenance of normocalcemia, adequate intestinal calcium absorption, bone turnover, and mineral homeostasis throughout the reproductive cycle—just as it is required in the nonlaying bird or nonpregnant, nonlactating mammal. In the avian species, 1,25-$(OH)_2D$ is required for ovulation and shell formation, transfer of calcium from the egg shell across the chorioallantoic membrane to the fetal circulation, and maintenance of fetal serum calcium, bone metabolism, and mineral homeostasis. In the mammalian species, 1,25-$(OH)_2D$ is required for normal ovulation, normal fetal and neonatal bone metabolism, milk production, and maintenance of normocalcemia and mineral homeostasis in the neonate.

In addition to the regulation of mineral metabolism, there is a growing excitement about the influence of 1,25-$(OH)_2D$ on cellular differentiation in tissues and cells other than the classical targets of vitamin D. There is no doubt that 1,25-$(OH)_2D$ can stimulate differentiation in numerous cells *in vitro*. To what extent these effects occur *in vivo*, however, remains to be determined. Relevant to this issue is the observation that, in the absence of 1,25-$(OH)_2D$, embryogenesis (rat and chick) and neonatal development (rat) can proceed in such a way as to produce, for the most part, viable, normal appearing offspring. The classical effects of 1,25-$(OH)_2D$ deficiency are obvious in these animals but there are no other apparent major abnormalities. 1,25-Dihydroxyvitamin D may influence cell differentiation in many tissues, but the magnitude of these effects and their importance for development and maintenance of homeostasis remain to be established.

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