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ORIGINAL ARTICLE

Comparative Analysis of Serum Calcium, Vitamin D, and Parathyroid Hormone in Pregnant and Non-Pregnant Arabian Mares

Farnaz Pouriayevali [1], Maryam Karimi-Dehkordi [2*], Majid Gholami-Ahangaran [2]

[1] *Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran*
[2] *Department of Veterinary, Shk.C., Islamic Azad University, Shahrekord, Iran*

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***Correspondence**

Author's Email:
ma_karimivet58@iau.ac.ir

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Abstract

This study aimed to investigate serum concentrations of calcium, vitamin D [25-hydroxyvitamin D3; 25(OH)D3], and parathyroid hormone (PTH) in Arabian mares in Iran, establish reference intervals for these parameters, and assess the potential effects of pregnancy and parity. Blood samples were collected from 30 clinically healthy Arabian mares, divided into three groups based on pregnancy and parity: non-pregnant mares (n = 10), pregnant mares in their first to third parity (n = 10), and pregnant mares in their sixth to sixteenth parity (n = 10). Serum calcium was measured using a colorimetric method, while vitamin D and PTH concentrations were determined using enzyme-linked immunosorbent assay (ELISA). Data were analyzed using one-way ANOVA, with $P < 0.05$ considered statistically significant. Mean serum concentrations of calcium, vitamin D, and PTH in all mares were 11.06 ± 0.89 mg/dL, 6.15 ± 1.2 ng/mL, and 24.57 ± 17.38 pg/mL, respectively. Pregnancy did not significantly affect serum concentrations of calcium, vitamin D, or PTH ($P > 0.05$). Parity had no significant effect on serum concentrations of calcium, PTH, or vitamin D (6.87 ± 1.47 ng/mL in mares in their sixth to sixteenth parity vs. 5.77 ± 0.73 ng/mL in mares in their first to third parity, $P = 0.06$). Serum calcium, vitamin D, and PTH concentrations remain relatively stable throughout pregnancy in Arabian mares, reflecting effective homeostatic regulation. Parity did not have a significant impact on these parameters. These results provide valuable reference data for monitoring mineral metabolism and guiding nutritional management in breeding mares.

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Introduction

Minerals and vitamins play essential roles in the normal functioning of the animal body (1). Among the minerals, calcium is the most abundant element in the animal body (2). It is also an essential constituent of the skeleton and teeth, and approximately 99% of total body calcium is found in bone and teeth (3, 4). The remaining calcium is distributed in blood in the two forms: non-ionized (protein-bound, chiefly to albumin), and ionized (free), the latter representing approximately 50% of the plasma calcium in horses. Furthermore, calcium is crucial to muscle contraction, exocrine secretion, calcified tissue formation, enzyme activity regulation, the permeability and excitability of the plasma membrane, neurotransmission, and signal transduction pathways (5-7). Calcium homeostasis is controlled by vitamin D [25-hydroxyvitamin D_3; $25(OH)D_3$], parathyroid hormone (PTH), and calcitonin (8).

In comparison with humans and other mammals, horses possess different characteristics in calcium homeostasis, including high ionized and total calcium concentrations as well as increased renal excretion and intestinal absorption of calcium (9). The calcium requirement in horses relies on physical activity, physiologic status, and age. For example, horses at maintenance should receive 20 to 25 mg of calcium/kg/d; whereas, adult horses should absorb approximately 40 mg of calcium/kg/day. In addition, lactating mares and growing horses require 50 to 75 g of calcium/day, whereas pregnant mares need 50 to 60 g of calcium/d (10).

Vitamin D is a fat-soluble vitamin and a member of the steroid hormone family that recognized as an essential nutrients for good health (11). Various biological effects of vitamin D have been proven by numerous studies. Classically, it plays a central role in the regulation of phosphorus and calcium metabolism. It also has a non-classic role in affecting immune regulation, cell proliferation, apoptosis, differentiation, neurogenesis, and genome stability (12, 13). Vitamin D increases the expression of genes associated with the trans-epithelial transport of phosphorus and calcium, which results in improvements in phosphorus and calcium absorption in the intestine and their reabsorption in the kidneys (9).

Vitamin D deficiency is highly prevalent worldwide (14). Recent studies have shown that vitamin D deficiency is associated with a wide range of chronic diseases, including cardiovascular disease, cancer, diabetes mellitus, bone metabolic disorders, depression, autoimmune diseases, chronic pain, and infectious diseases (15). Vitamin D exists in several forms, of which vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) are the two major forms (16). Both vitamin D_3 and vitamin D_2 are metabolized in the liver to 25-hydroxyvitamin D_3 [$25(OH)D_3$], which is the primary biomarker of vitamin D status. In the kidney, $25(OH)D_3$ is further hydroxylated to form the biologically active metabolite 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which mediates most of the physiological effects of vitamin D (17).

Compared with humans and other domestic animals, horses have markedly lower concentrations of vitamin D metabolites. Nevertheless, physical activity decreases the serum levels of $25(OH)D_3$ in healthy horses (9).

PTH, also known as parathyrin or parathormone, is a polypeptide hormone synthesized and secreted by the parathyroid glands. It plays an essential role in bone metabolism and regulates extracellular phosphate and calcium homeostasis (18). PTH prevents phosphate reabsorption and also increases calcium reabsorption in the kidney tubule (19). Additionally, PTH enhances intestinal calcium absorption by increasing the synthesis of 1,25-dihydroxyvitamin D in the kidneys (20).

The Arabian horse is a breed renowned for its speed, endurance, beauty, intelligence, gentle temperature, and strong bones. Moreover, the Arabian horse is one of the oldest breeds in the world, dating back to approximately 4,500 years (21). Among all horse breeds around the globe, the Arabian horse can be easily recognized by its characteristic head shape and high tail carriage (22).

In recent years, increasing attention has been paid to the assessment of biomarkers and general health in Iranian horse breeds. Serum oxidative status has been evaluated in Arabian and Dareh-Shour horses, revealing a stable profile of oxidative stress indicators (23). Further studies on Dareh-Shour mares have reported normal ranges of reproductive hormones and their associations with pregnancy rates, which can be highly useful for monitoring reproductive health in this breed (24). These findings highlight the importance of investigating physiological, metabolic, and reproductive factors in native Iranian horse breeds.

To our knowledge, no previous studies have assessed serum levels of calcium, vitamin D, and parathyroid hormone (PTH) in Arabian mares in Iran. This study aimed to evaluate these parameters, establish reference intervals, and investigate the effects of pregnancy status and parity on their concentrations.

Materials and Methods

Animals and Blood Sampling

All blood samples were collected from 30 healthy Arabian mares in Chaharmahal-va-Bakhtiari Province (31°58′N 50°29′E), Iran. Based on pregnancy status and parity, the mares were divided into in three groups non-pregnant mares (n = 10), pregnant mares in their first to third parity (n= 10), and pregnant mares in their sixth to sixteenth parity (n = 10). All the mares were clinically healthy and in a stable physical condition. Moreover, they did not have any history of diseases and had not received any treatments for at least one month before the study. None of the horses performed work throughout the blood sample collection period. They were also kept in individual stalls for 23 h/day under natural indoor temperature and a natural photoperiod, and were allowed access to sunshine for 1 h/day. The animals were fed on grass and grass hay and had access to grazing for 1 h/day. Furthermore, they had ad libitum access to water all the time.

Blood sampling was conducted in Chaharmahal-va-Bakhtiari Province, Iran. According to a study conducted by Piccione et al. (2008), the serum concentrations of vitamin D in horses are found to be lower in the morning compared with those in the early afternoon (25) . Thus, all blood samples from the horses were taken by jugular venipuncture in the morning. The blood samples were placed in tubes with no additives, allowed to clot at room temperature for 1 h, centrifuged at 4,000 × g for 10 min at 37 °C, and stored at -20 °C until further analysis.

Serum Analysis

The samples were separately evaluated for the serum concentration of calcium by colorimetric methods (25) by applying commercially available diagnostic kits (Pars Azmun Co. Ltd, Karaj, Iran) and an auto-analyzer (BT 3000, Japan).

Besides, enzyme-linked immunosorbent assay (ELISA) technique (ELISA, BioTek ELX800 reader, BioTek ELX50 washer, Winooski, Vermont, United States) was used to measure the serum concentrations of vitamin D (ELISA Kit, Monobind Inc., Lake Forest, CA, USA) and PTH (horse PTH ELISA Kit, MyBioSource, Inc., USA) in the plasma samples (9).

Data Analysis

Absolute concentrations were used to obtain the results from the serum calcium, vitamin D, and PTH analyses. All values were expressed as mean ± SEM. The data were normally distributed ($P < 0.05$), and one-way repeated measures analysis of variance (ANOVA) was employed to define significant differences. A P value of < 0.05 were considered statistically significant for all statistical comparisons in this study.

Ethical Statement

All experimental procedures involving animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Islamic Azad University, Shahrekord Branch, under the approval number IR.IAU.SHK.REC.1403.094. The study was conducted in accordance with relevant guidelines and regulations for the ethical use and care of animals in research.

Results

The mean (± SD) serum concentrations of calcium, vitamin D and PTH in all mares (n = 30) were 11.06 ± 0.89 mg/dL, 6.15 ± 1.2 ng/mL, and 24.57 ± 17.38 pg/mL, respectively. Table 1 presents the mean (± SD) serum levels of Ca in the three groups of mares. As shown in Table 1, the non-pregnant mares had the highest concentration of calcium (11.47 ± 0.6 mg/dL). The effect of pregnancy on serum calcium was compared between the two groups of non-pregnant (n = 10) and pregnant (n = 20) mares. The non-pregnant mares had higher levels of calcium than the pregnant ones. The results also showed that the effect of pregnancy on serum calcium concentrations was not significant ($P = 0.08$). The effect of parity on the pregnant mares was not significant ($P = 0.8$), although the pregnant mares in their sixth to sixteenth parity had higher calcium levels than those in their first to third parity.

The mean (± SD) serum concentrations of vitamin D in the three groups of Arabian mares are illustrated in Table 2. The results in this table revealed that the pregnant mares in their sixth to sixteenth parity showed the highest level of vitamin D among the three groups (6.87 ± 1.47 ng/mL). Furthermore, the effect of pregnancy on serum vitamin D was studied by the comparison of the vitamin D concentrations of non-pregnant mares (n = 10) with pregnant mares (n = 20). Pregnancy had no significant effect on serum vitamin D concentrations ($P = 0.2$). However, the pregnant mares had higher levels of vitamin D than the non-pregnant ones. To investigate the effect of parity on serum vitamin D concentrations, the pregnant mares in their first to third parity were compared with those in their sixth to sixteenth parity. Serum vitamin D concentrations in the pregnant mares in their sixth to sixteenth parity were significantly higher than those in their first to third parity. These results also showed a significant effect of parity on serum vitamin D concentrations in the pregnant Arabian mares ($P = 0.04$).

Table 1. Effects of pregnancy and parity on serum calcium concentrations in Arabian mares (mg/dL)

Horses	N	Calcium	*P* value
Non-pregnant mares	10	11.47 ± 0.6	0.6
Pregnant mares in their first to third parity	10	10.83 ± 0.89	
Pregnant mares in their sixth to sixteenth parity	10	10.9 ± 1.08	
Effect of pregnancy	**N**	**Calcium**	***P* value**
Non-pregnant mares	10	11.47 ± 0.6	0.08
Pregnant mares	20	10.87 ± 0.96	
Effect of parity	**N**	**Calcium**	***P* value**
Pregnant mares in their first to third parity	10	10.83 ± 0.89	0.8
Pregnant mares in their sixth to sixteenth parity	10	10.9 ± 1.08	

The serum concentrations of PTH in three groups of the Arabian mares are shown in Table 3. The highest mean serum PTH concentrations was observed in the pregnant mares in their first to third parity (26.46 ± 21.61 pg/mL). Additionally, the PTH concentrations of non-pregnant mares (n = 10) and pregnant mares (n = 20) were compared to evaluate the effect of pregnancy on serum PTH concentrations in the mares. The pregnant mares showed a higher concentration of PTH than non-pregnant mares. However, the effect of pregnancy on serum PTH concentrations in the mares was not significant (P = 0.9). Besides, the effect of parity on serum PTH concentrations in the mares was investigated by the comparison of the PTH concentrations in the pregnant mares in their first to third parity and those in their sixth to sixteenth parity. Pregnant mares in their first to third parity had slightly higher serum PTH concentrations than those in their sixth to sixteenth parity; however, this difference was not statistically significant (P = 0.06).

Table 2. Effects of pregnancy and parity on serum vitamin D concentrations in the Arabian mares (ng/mL)

Horses	N	Vitamin D	*P* value
Non-pregnant mares	10	5.82 ± 1.04	0.3
Pregnant mares in their first to third parity	10	5.77 ± 0.73	
Pregnant mares in their sixth to sixteenth parity	10	6.87 ± 1.47	
Effect of pregnancy	**N**	**Vitamin D**	***P* value**
Non-pregnant mares	10	5.82 ± 1.04	0.2
Pregnant mares	20	6.32 ± 1.26	
Effect of parity	**N**	**Vitamin D**	***P* value**
Pregnant mares in their first to third parity	10	5.77 ± 0.73	0.06
Pregnant mares in their sixth to sixteenth parity	10	6.87 ± 1.47	

Table 3. Effects of pregnancy and parity on serum PTH concentrations in the Arabian mares (pg/mL)

Horses	N	PTH	*P* value
Non-pregnant mares	10	23.97 ± 20.07	0.5
Pregnant mares in their first to third parity	10	26.46 ± 21.61	
Pregnant mares in their sixth to sixteenth parity	10	23.27 ± 9.86	
Effect of pregnancy	**N**	**PTH**	***P* value**
Non-pregnant mares	10	23.97 ± 20.07	0.9
Pregnant mares	20	24.86 ± 16.43	
Effect of parity	**N**	**PTH**	***P* value**
Pregnant mares in their first to third parity	10	26.46 ± 21.61	0.06
Pregnant mares in their sixth to sixteenth parity	10	23.27 ± 9.86	

Discussion

The present study aimed to evaluate the effects of pregnancy and parity on serum concentrations of calcium, vitamin D, and PTH in healthy Arabian mares. Understanding these physiological parameters is critical for managing reproductive health and mineral homeostasis in equine species.

In the present study, serum calcium concentrations in healthy Arabian mares ranged from 10.83 ± 0.89 mg/dL in pregnant mares of first to third parity to 11.47 ± 0.6 mg/dL in non-pregnant mares. Pregnant mares in their sixth to sixteenth parity showed slightly higher serum calcium concentrations (10.9 ± 1.08 mg/dL), although neither pregnancy (P = 0.08) nor parity (P = 0.8) had a significant effect on serum calcium. These values are generally consistent with those reported by Effati et al. (2018) in Caspian horses, where female horses had a mean serum calcium concentration of 12.66 ± 0.44 mg/dL, with significantly higher concentrations observed in summer (14.25 ± 0.38 mg/dL) compared with winter (11.50 ± 0.38 mg/dL), and slightly higher calcium in horses older than 3 years (13.17 ± 0.45 mg/dL) compared with younger horses (≤ 3 years: 12.59 ± 0.42 mg/dL) (26).

These findings suggest that maternal calcium homeostasis is efficiently maintained during gestation, likely due to adaptive physiological mechanisms and homeostatic regulation (27, 28), and are consistent with previous studies reporting minimal changes in total serum calcium during equine gestation (25, 29). Moreover, environmental and intrinsic factors may contribute to modest variations in calcium levels, as highlighted by Effati et al. (2018) and Alemi et al. (2025), who reported seasonal differences and minor effects of age, breed, and other factors on serum calcium in horses (26, 30). Collectively, these results indicate that serum calcium in equines remains within a relatively narrow physiological range under both physiological and environmental variations, aligning with the stable levels observed in both pregnant and non-pregnant Arabian mares in the present study.

In the present study, serum vitamin D concentrations in healthy Arabian mares ranged from 5.77 ± 0.73 ng/mL in pregnant mares in their first to third parity to 6.87 ± 1.47 ng/mL in pregnant mares with the sixth to sixteenth parity, while non-pregnant mares had a mean concentration of 5.82 ± 1.04 ng/mL. Pregnancy had no significant effect on serum vitamin D concentrations (P = 0.2); however, the number of parities showed a nearly significant effect, indicating that multiparous mares tended to have higher concentrations of this vitamin (P = 0.06).

The serum vitamin D concentrations observed in this study differed from those reported in horses of other breeds and regions. For example, Salehi-Ardakani et al. (2023) reported a mean concentration of 27.33 ng/mL in Arabian mares from Yazd Province (31), whereas Dosi et al. (2023) found much lower values (6.32 ng/mL) in Thoroughbred horses (32). Similarly, Azarpeykan et al. (2016) reported concentrations below 4 ng/mL in New Zealand (33). In contrast, Pozza et al. (2014) reported ranges of 18.4–30.5 ng/mL in Thoroughbreds in Thailand and 14.3–37.2 ng/mL in Thoroughbreds in the United States (34), which are comparable to the findings of the present study. Moreover, the absolute values in the current study were higher than those reported by Effati et al. (2018) in Caspian horses (females: 2.26 ± 0.28 ng/mL; summer: 2.48 ± 0.27 ng/mL; winter: 1.66 ± 0.28 ng/mL; > 3 years: 2.24 ± 0.28 ng/mL; ≤ 3 years: 1.91 ± 0.27 ng/mL) (26).

These differences in reported vitamin D levels among studies may be attributed to a combination of intrinsic and extrinsic factors, including breed, age, skin color, dietary intake of vitamin D, supplementation, geographical latitude, and environmental conditions such as intensity and duration of sunlight exposure (34). Since vitamin D is mainly synthesized in the skin in response to ultraviolet (UV) light, reduced sunlight exposure in certain seasons or management practices such as housing mares in covered stables can decrease serum vitamin D concentrations (35). Differences in assay methods and sample handling among studies may also contribute to the observed variability.

Overall, these findings indicate that although pregnancy and parity number do not have a substantial effect on vitamin D status, a combination of intrinsic and extrinsic factors can influence serum vitamin D concentrations in horses. These results highlight the importance of considering environmental and management factors when evaluating vitamin D status to ensure proper calcium balance and bone health. In line with the importance of management factors, Karimi-Dehkordi et al. (2023) also reported that the Bio-Equine probiotic can influence certain blood parameters related to the metabolic status of Dare-shoor mares (36). Although some studies have reported an association between age and vitamin D status in horses (31, 34), no significant relationship was observed in the present study. Since parity was used as an indirect indicator of age, it can be inferred that increasing age (as reflected by a higher number of parities) does not have a notable effect on vitamin D concentrations, particularly as the diet of mares older than six months mainly consists of grass and hay.

In the present study, serum PTH concentrations in healthy Arabian mares ranged from 23.27 ± 9.86 pg/mL in pregnant mares in their sixth to sixteenth parity to 26.46 ±

21.61 pg/mL in pregnant mares in their first to third parity, while non-pregnant mares had a mean concentration of 23.97 ± 20.07 pg/mL. Neither pregnancy (P = 0.9) nor parity (P = 0.6) had a significant effect on PTH concentrations, indicating maintenance of calcium homeostasis during gestation. Minor differences in PTH among mares of different parity were not statistically significant, and this stability aligns with previous reports showing tightly regulated serum PTH across seasons, age groups, and breeds in horses (26, 30).

These results are in agreement with the findings of Effati et al. (2018) in Caspian horses, where mean PTH in females was 65.05 ± 10.48 pg/mL with seasonal variations (summer: 73.23 ± 7.72 pg/mL; winter: 70.16 ± 7.76 pg/mL), and slightly higher concentrations in older horses (> 3 years: 83.14 ± 10.79 pg/mL) compared with younger horses (≤ 3 years: 60.25 ± 10.13 pg/mL). In that study, the effects of sex, season, age, and their interactions on serum PTH levels were not significant, and all values remained within the normal range reported for horses (26). Although absolute PTH values in the present study were lower than those reported by Effati et al. (2018), these differences may be attributed to demographic and physiological factors, including breed, age, and coat color of the mares.

Such observations highlight the robustness of PTH regulation in maintaining calcium homeostasis during gestation. This tight regulation ensures proper calcium metabolism and bone health, emphasizing the effectiveness of the parathyroid axis under both physiological and environmental variations.

Conclusion

Overall, these findings indicate that in healthy Arabian mares, pregnancy does not markedly alter serum calcium, vitamin D, or PTH concentrations, suggesting that maternal mineral homeostasis is robustly maintained during gestation. However, parity may have a modest effect on vitamin D metabolism, potentially reflecting adaptive changes in repeated pregnancies. These results are clinically relevant for managing nutritional supplementation and monitoring mineral status in breeding mares to support optimal reproductive performance and fetal development.

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Authors' Contributions

Farnaz Pouriayevali: Investigation, formal analysis, writing – original draft preparation. **Maryam Karimi-Dehkordi:** Conceptualization, supervision, project administration, writing – review & editing, corresponding author. **Majid Gholami-Ahangaran:** Methodology, validation, writing – review & editing. All authors read and approved the final manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

All procedures of this study were conducted in compliance with ethical principles in animal research and were approved by the Ethics Committee of the Faculty of Veterinary Medicine (ethics code: IR.IAU.SHK.REC.1403.094).

Conflict of Interest

The authors declare that they have no known competing financial or non-financial interests that could have appeared to influence the work reported in this paper.

Consent for Publication

Not applicable.

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