Study of Hypocalcaemia in pregnant Ewes

Omar Al-Thani Sh. Saed Al-Bayati
Collage of veterinary medicine / University of Tikrit

Abstract:

Experiment was conducted from 1/1/2015-1/7/2015 to study the clinical signs and biochemical changes including calcium, phosphor, magnesium and glucose in pregnant ewes in comparison with control (non pregnant ewes). The study was included 30 alawassi ewes (24 pregnant and 6 control (dry or non-pregnant ewes)) and through the using of vaginal sponges to unify estrus, pregnancy and coincided on the same day. The study noticed clinically, clinical signs that appeared on the pregnant ewes at the end of the fifth month of pregnancy before, during and after parturition. In all pregnant ewes, 24 ewes showed the clinical signs hypocalcemia, included depression (100%), rapid respiration (100%), tachy cardia (83.3%), muscular tremor (66.6%), muscular weakness (66.6%), anorexia (66.6%), teeth grinding (62.5), hypothermia (62.5%), normal temperature (37.5%), Stiff and uncoordinated movement (16.6%), Ruminal stasis and tympany (12.5 %) and sternal recumbency (8.3%). It was study observed that ewes pregnant with twin lambs, 9 of 24 ewes, 8 of them, (88.9%) shawed signs of hypocalcemia while pregnant with single lamb was 15 out of 24 ewes, 8 of them only, (53.3%) showed signs of Hypocalcemia. Also noticed in this study that pregnant ewes in age (≥ 3) years 12 ewes 10 of them, (83.3%) shawed signs of hypocalcaemia while pregnant ewes in age between (1-3) years was 12, 6 (50%) showed signs of Hypocalcemia. The levels of Ca, P, Mg and Glucose in serum in the period of first, second and third month of gestation, were within the normal ranges in pregnant ewes when differentiated with the control (dry ewes). While the calcium level in serum of the pregnant ewes (clinically sick) in the final fourth and fifth months of pregnancy was significantly lower than the Ca level in pregnant ewes (clinically normal) and control (dry ewes). However the glucose levels in serum of pregnant ewes (clinically sick) in the end of fifth month of gestation, was significantly ($P < 0.05$) higher than glucose levels in pregnant ewes (clinically normal) and control (dry ewes).

دراســـة نقص الكالسيــوم فـي النعــاج الحـوامــل

عمرالثاني شريف سعيد البياتي
كليــة الطب البيطــري / جامعــة تكــريت

الخلاصــة :

اجريت التجربة من 2015/1/1 ولغاية 2015/7/1 لدراسة العلامات السريرية والتغيرات البيوكيميائية والتي شملت الكالسيوم والفوسفور والمغنيسيوم والجلوكوز في النعاج الحوامل ومقارنتها مع مجموعة السيطرة (النعاج غير الحوامل) اجريت هذه الدراسة على 30 نعجة عواسية (24 نعجة حامل) والسيطرة (6 نعاج غير حامل)، من خلال استخدام الاسفنجات المهبلية لتوحيد دورات الشبق والحمل والولادات في النعاج. وقد لوحظ خلال الدراسة اهم العلامات السريرية التي ظهرت على النعاج الحوامل (24 نعجة) في نهاية الشهر الخامس من الحمل قبل وأثناء وبعد الولادة والتي شملت الخمول (100%)، التنفس السريع (100%)، زيادة في ضربات القلب (83.3%)، رعاش عضلي (66.6%)، ضعف العضلات (66.6%)، وفقدان الشهية (66.6%)، طحن الاسنان (62.5%)، انخفاض حرارة الجسم (62.5%)، درجة الحرارة الطبيعية (37.5%)، الحركة او السير غير المنتظم ((16.6%)، ركود الكرش والنفاخ (12.5%) والاستلقاء القصي للحيوان (8.3%). كما لوحظ ان النعاج التي كانت حامل بالتوأم والتي بلغ عددها 9 من مجموع 24 نعجة حامل، ان 8 منها وبنسبة (88.9%) ظهر عليها علامات نقص الكالسيوم، في حين النعاج التي كانت حامل بجنين واحد والتي عددها 15 نعجة من مجموع 24 نعجة حامل، ان 8 منها فقط وبنسبة (53%) ظهرت عليها علامات نقص الكالسيوم، كما لوحظ بان النعاج الحوامل والتي اعمارها (≤ 3) سنوات والذي عددها 12 نعجة، كانت 10 منها وبنسبة (83.3%) ظهرت عليها علامات نقص الكالسيوم في حين النعاج التي اعمارها تتراوح مابين (1-3) سنوات، 6 منها فقط من مجموع (12) نعجة وبنسبة (50 %) ظهرت عليها علامات النقص بالكالسيوم. وتبين ان معدل مستويات الكالسيوم، الفسفور، المغنيسيوم والكلوكوز في المصل في الفترة من الشهر الاول والثاني والثالث من الحمل كانت ضمن النسب الطبيعية في النعاج الحوامل عند مقارنتها مع نعاج السيطرة (غير الحامل) في حين لوحظ حصول انخفاض معنوي لمعدلات نسب الكالسيوم في النعاج الحوامل (المرضى سريرياً) خلال الشهر الرابع والخامس من الحمل عند مقارنتها مع النعاج الحوامل (الطبيعية سريرياً) ونعاج السيطرة (غير الحامل). كما لوحظ حصول ارتفاع معنوي في معدلات

نسب الجلوكوز في مصل دم النعاج الحامل (المرضى سريرياً) في نهاية الشهر الخامس من الحمل عند مقارنتها مع النعاج الحامل (الطبيعية سريرياً) ونعاج السيطرة (غير الحامل).

Introduction

Hypocalcemia (milk fever, parturient paresis, parturient hypocalcemia, parturient apoplexy) is production diseases of cattle, sheep, and goats occurring around the time of parturition and characterized by progressive neuromuscular dysfunction with flaccid paralysis, circulatory collapse, depression of consciousness and ultimately shock and death. The main cause of disease is a depression of ionized calcium in serum and tissues (Pickard, 2000; Overton and Waldon, 2004).

In ewe, the disease commonly occurs in outbreaks in groups of ewes exposed to forced exercise, long-distance transport, sudden deprivation of food and grazing on oxalate-containing plants or green cereal crops. These circumstances commonly precipitate outbreaks of hypocalcemic paresis in ewes; mature ewes are the most susceptible, particularly in the period from 6 weeks before to 10 weeks after lambing, up to 25 % of the flock may be affected at one time, The disease also occurs in young sheep up to about 1 year old, especially when they graze green oats, but also when pasture is short in winter and spring (Tindall, 1986).

Ewe is considered to be hypocalcemic when a significant decline in plasma Ca concentration occurs (Horst *et al*., 1994). A complex endocrine regulating system is in place to ensure that extracellular Ca concentration remains within a narrow range (10 – 13 mg /dL), (Goff *et al*., 1991). There are varying degrees of hypocalcemia, but most ewes experience some level of hypocalcemia around parturition (Goff, 2000). However, significant additional costs are usually endured because a hypocalcemic ewe has an increased risk of developing other problems such as pregnancy toxemia and mastitis (Overton and Waldon, 2004).

Hypophosphatemia and variations in levels of serum magnesium also occur in hypokalemia and have secondary roles. The incidence of milk fever generally increased with parity and with higher levels of milk production. Older dairy ewe with a history of parturient paresis during a previous lactation and high-producing ewe are at highest risk for developing parturient paresis, Hypokalemia is a metabolic disorder of calcium homeostasis that affects about 2 to 6% of postpartum ewe (Radostits, *et al*., 2007). Hypocalcaemia can depress glucose production and enhance insulin resistance of tissues (Schlumbohm, *et al*., 1997).

Calcium is tightly regulated in mammals because of the critical role of calcium ion concentrations in many physiological functions. Sudden calcium outflow occurs most commonly at the time of the initiation of lactation. The calcium demand associated with colostral production, exceeds the total prepartum calcium requirements (mineralization of fetal skeleton). (Howard and Marsh, 1999).

As mentioned earlier several factors have been consistently associated with increased incidence of hypokalemia, including parturition and initiation of lactation, advancing age, breed, and diet. Calcium is an important physiological cation, Calcium salts maintain the integrity of the skeleton structure, and calcium ions in intracellular and extracellular fluids are instrumental in controlling a large number of biochemical processes. While intracellular calcium ions are needed in the activity of a large number of enzymes and are also involved in conveying information from the surface to the interior of the cell, extracellular calcium ions are necessary for neuro-muscular excitability, blood clotting and hormonal secretion among many other functions. For these important biochemical roles to be accomplished, extracellular and intracellular concentrations of calcium are maintained within a narrow range. (Griffin & Ojeda, 1996).

Hypocalcemia can be a problem in dairy ewes. It usually occurs shortly before or after parturition and is a result of low concentrations of serum calcium. Some cases also are complicated by hypophosphatemia

and hypermagnesemia or hypomagnesemia. Ewes appear most susceptible in late gestation and early lactation, particularly when experiencing some sort of stress (hauling, predator attack, no feed). Sheep may succumb to hypocalcemia 6 weeks before to 10 weeks after parturition. The greatest demand for calcium for the non-dairy animal occurs 3 to 4 weeks before parturition in females with more than one fetus, as a result of the calcification of fetal bones. High intake of calcium, phosphorus, or some cations (potassium, sodium) decreases the production of parathyroid hormones. During decreased parathyroid function, less 1,25 dihydroxycholecalciferol is produced. This results in lowered absorption and mobilization of calcium from the intestines and bones. Low dietary calcium or increased amounts of dietary anions enhances the production and release of parathyroid hormones. (Horst *et al*., 1994; Pugh, 2002).

And the lack of studies and promote changes the clinical and biochemical results associated with hypocalcaemia in pregnant ewes, this study was carried out to achieve the following objectives:

- To study the most important clinical signs that appear on the ewes that suffer hypocalcemia in the last month of pregnancy.
- To determine level of calcium, phosphorus, magnesium and glucose in blood serum in pregnant ewes.
- To study of the relationship between Hypocalcemia, Ewes age and number of the fetus in pregnant ewes.

Materials & Methods:

This study was conducted during the period from the beginning of the month of January / 2015 and lasted until the end of June / 2013, To Study the clinical signs and biochemical changes which including calcium, phosphorus, magnesium and glucose concentration in pregnant ewes in comparison with control (dry or non pregnant ewes). The process was held on 30 Awassi ewes (24 pregnant and 6 control (dry ewes) using of vaginal sponges to unify estrus in ewes and pregnancy coincided in the same day. After that, blood samples were collected at the end of each month of pregnancy (5 months) of pregnant and control ewes, and have been registered as clinical signs that appeared on the ewes at the end of the fifth month of pregnancy before, during and after parturition.

Unification of estrus and pregnancy in ewes:

unification of oestrus and pregnancy in ewes has obtained through placing vaginal sponges saturated with progesterone hormone in the vagina of ewe and left for a period of 12 days then sponge was pulled from vagina and ewes were injected with Gonadotropin-releasing hormone (GnRH), then ewes were inseminated naturally.

Samples collection:

Blood samples were from the jugular vein using syringes, plastic-use one after sterilize the area with 70% ethyl alcohol, where it 10 ml of blood from each animal was withdrawn and placed in test tubes free from objection coagulation and stored at 4 ^{o}C overnight, then the serum was separated. for the purpose of biochemical test. and preserved in the refrigerator, while kept at 20 ^{o}C until testing them (Coles, 1986).

Clinical examination of pregnant ewes:

Prepared special forms for recording clinical signs appeared on the pregnant ewes in times of parturition.

Biochemical tests for serum:

Was measured concentration of serum calcium, phosphorus, magnesium and glucose where determine using solutions standard readymade Kits to serum according to the manufacturer's instructions using a spectroscope photosynthesis (Meyer and Harvey, 2004).

Results & Discussion

Clinical examination results:

In all pregnant ewes, 24 ewes showed the clinical signs of hypocalcemia. As shown in (Table 1) included depression (100 %), rapid respiration (100 %), tachycardia (83.3 %), muscular tremor (66.6 %), muscular weakness

(66.6 %), anorexia (66.6 %), teeth grinding (62.5), hypothermia (62.5 %), normal temperature (37.5 %), Stiff and uncoordinated movement (16.6 %), ruminal stasis and tympany (12.5 %) and sternal recumbency (8.3). Typical signs observed were in accordance with previously described findings (Scott, 1995; Cockcroft and Whiteley, 1999; Radostitis *et al*., 2007). Similar clinical signs were reported by other authors (Jensen and Swift, 1982; Pickard, 2000), but teeth grinding (62.5%) were atypical findings in the present study's hypocalcemic ewes, which is in contrast to previous reports (Woldemeskel *et al.* 2012), It was documented in ewes with combined hypocalcemia and hypomagnesemia, Teeth grinding is related more to hypomagnesemia than to hypocalcemia.

Table 1: Clinical signs that appeared in pregnant ewes in late stage of pregnancy.

Clinical Signs Manifested	No. of ewes showing the signs	Percentage (%)
Depression (varying degree)	24	100
Rapid breathing	24	100
Tachycardia	20	83.3
Muscular tremor	16	66.6
Muscular weakness	16	66.6
Anorexia	16	66.6
Teeth grinding	15	62.5
Hypothermia	15	62.5
Normal temperature	9	37.5
Stiff and uncoordinated movement	4	16.6
Ruminal stasis and tympany	3	12.5
Sternal recumbency	2	8.3

Relationship of hypocalcaemia with number of fetus and age of pregnant ewes.

It was observed that ewes pregnant with twin lambs was (9 ewes), 8 of them (88.9%) showed signs of hypocalcemia while pregnant ewes pregnant with single lamb (15 ewes), 8 of them (53.3%) showed signs of hypocalcemia.

We also noticed through this study, that pregnant ewes in age (≥ 3) years (12 ewes) 10 of them, (83.3%) shawed signs of hypocalcaemia, while pregnant ewes in age (1-3) years were 12 ewes, 6 of them, (50%) showed signs of hypocalcemia.

The ewes carrying twin lambs and the pregnant ewes in age of more than 3 years are more infected with hypocalcaemia, this is in agreement with (Radostitis *et al.*, 2007), pregnant ewes with more than one fetus need more calcium ratio for the development of the fetus and therefore be more affected with hypocalcemia. Older ewes are less able to respond to the Vitamin D and will take longer time to adapt intestinal calcium absorption mechanisms to meet lactational demands for calcium (Woldemeskel, *et al*., 2012; Radostitis *et al.*, 2007).

Table 2: Results of relationship between number of fetus and the age of ewes with the hypocalcemia occurs in pregnant ewes.

Variables	Single lambs (n=15)	Twin lambs (n= 9)	Age (1-3) years (n=12)	Age (≥ 3) years (n= 12)
Pregnant ewe (Clinically Sick)	8 (53.3%)	8 (88.9%)	6 (50%)	10 (83.3%)
Pregnant Ewes (Clinically normal)	7 (46.7)	1(11.1%)	6 (50%)	2 (16.7%)

Biochemical examination results:

- **In first month of gestation:**

The mean serum Ca, P, Mg and Glucose values were within the normal ranges in pregnant ewes when compared with the control (dry ewes). Table (3).

Table 3: Biochemical examination (Ca, Mg, P and glucose) in Control (dry ewes) and Pregnant ewes in first month.

Serum constituents	pregnant ewe (Clinically Sick) (n =16)	Pregnant Ewes (Clinically normal) (n = 8)	Control (dry Ewes) (n = 6)
Ca (mmol/l)	2.68 ± 0.41	2.75 ± 0.40	2.60 ± 0.12
P (mmol/l)	2.05 ± 0.14	2.20 ± 0.14	2.10 ± 0.09
Mg (mmol/l)	1.18 ± 0.08	1.15 ± 0.30	1.90 ± 0.30
Glucose (mmol/l)	3.55 ± 0.13	3.72 ± 0.12	3.70 ± 0.12

- **In second month of gestation:**

The mean serum Ca, P, Mg and glucose concentration were within the normal ranges in pregnant ewes in comparison with the control (dry ewes). Table (4).

Table 4: Biochemical examination (Ca, Mg, P and glucose) in Control (dry ewes) and pregnant ewes in second month.

Serum constituents	pregnant ewe (Clinically Sick) (n =16)	Pregnant Ewes (Clinically normal) (n = 8)	Control (dry Ewes) (n = 6)
Ca (mmol/l)	2.63 ± 0.24	2.80 ± 0.21	2.62± 0.13
P (mmol/l)	2.12 ± 0.10	2.21 ± 0.09	2.08 ± 0.18
Mg (mmol/l)	1.16 ± 0.08	1.11 ± 0.30	1.20 ± 0.30
Glucose (mmol/l)	3.40 ± 0.13	3.66 ± 0.08	3.59 ± 0.10

- **In third month of gestation:**

The mean serum Ca, P and Glucose concentration were within the normal ranges in pregnant ewes in comparision with the control (dry ewes). But the concentration of Mg was decreased in pregnant and control ewes in this month in comparison with first, second, fourth and fifth month of gestation period. Table (5).

Table 5: Biochemical examination (Ca, Mg, P and glucose) in Control (dry ewes) and pregnant ewes in third month.

Serum constituents	pregnant ewe (Clinically sick) (n =16)	Pregnant ewes (Clinically normal) (n=8)	Control (Dry ewes) (n = 6)
Ca (mmol/l)	2.36 ± 0.11	2.44 ± 0.05	2.59 ± 0.12
P (mmol/l	2.11 ± 0.14	2.23 ± 0.14	2.19 ± 0.09
Mg (mmol/l)	0.85 ± 0.08	0.90 ± 0.30	1.00 ± 0.30
Glucose (mmol/l)	3.50 ± 0.13	3.71 ± 0.12	3.7 ± 0.12

The mean serum Ca, P, Mg and glucose concentration in the period of first, second and third month of gestation, were within the normal ranges in pregnant ewes in comparison with the control (dry ewes), this is in agreement with previous observations noticed by different authors (Sweney and Cudderford, 1987; Littlejohn and Herbert, 1999), minerals concentration was decreased in late stages of gestation in pregnant ewes. But only the mean serum Mg concentration in the current study was non significantly decreased in all animals pregnant and control ewes in third month when differences with other months of gestation, this is in agreement with (Radostitis *et al*., 2007), Hypomagnesemia can also occur in animals grazing pastures (lush grass-dominant pasture), with adequate concentrations of magnesium but that contain high concentrations of potassium and nitrogen, which as detailed earlier, impair absorption of magnesium in the rumen and lead to hypomagnesemia. This corresponds to experimental animals which were fed on lush grass pasture in the third month of pregnancy.

- **In fourth month of gestation:**

The mean serum Ca concentration of the pregnant ewes (clinically sick) (1.33 ± 0.17 mmol/l) was significantly ($P < 0.05$) lower than the concentration for pregnant ewes (clinically normal) (2.37 ± 0.21mmol/l) and control (dry ewes) (2.64 ± 0.15 mmol/l), while, the mean serum magnesium and phosphorus concentration were within the normal range. (Table 6).

Table 6: Biochemical examination (Ca, Mg, P and glucose) in control (dry ewes) and pregnant ewes in fourth month.

Serum constituents	pregnant ewe (Clinically Sick) (n =16)	Pregnant ewes (Clinically normal) (n=8)	Control (Dry ewes) (n = 6)
Ca (mmol/l)	1.33 ± 0.17[a]	2.37 ± 0.21[b]	2.64 ± 0.15[b]
P (mmol/l)	2.04 ± 0.14	2.30 ± 0.14	2.21 ± 0.09
Mg (mmol/l)	1.04 ± 0.08	1.10 ± 0.30	1.19 ± 0.30
Glucose (mmol/l)	3.88 ± 0.13	3.79 ± 0.12	3.60 ± 0.12

[a,b] Different letters in a row show significant ($p < 0.05$) difference among the groups.

- **In fifth month of gestation:**

The mean serum Ca concentration of the pregnant ewe (clinically sick) (1.15 ± 0.3 mmol/l) was significantly (P < 0.05) lower than the concentration in pregnant ewe (clinically normal) (2.32 ± 0.23 mmol/l) and control (dry ewes) (2.78 ± 0.21mmol/l). but The mean serum glucose concentration of the pregnant ewe (clinically sick) (5.65 ± .13 mmol/l) was significantly (P < 0.05) higher than the concentration in pregnant ewe (clinically normal) (3.83 ± 0.12 mmol/l) and control (dry ewes) (3.67 ± 0.12 mmol/l). While, the mean serum magnesium and phosphorus were within the normal range. (Table 7).

Table 7: Biochemical examination (Ca, Mg, P and glucose) in Control (dry ewes) and pregnant ewes in fifth month.

Serum constituents	pregnant ewe (Clinically Sick) (n =16)	Pregnant ewes (Clinically normal) (n=8)	Control (dry ewes) (n = 6)
Ca (mmol/l)	1.15 ± 0.31[a]	2.32 ± 0.23[b]	2.78 ± 0.21[b]
Mg (mmol/l)	1.20 ± 0.08	1.13 ± 0.30	1.18 ± 0.30
P (mmol/l)	1.97 ± 0.14	2.09 ± 0.14	2.19 ± 0.09
Glucose (mmol/l)	5.65 ± 0.13[a]	3.83 ± 0.12[b]	3.67 ± 0.12[b]

[a,b] Different letters in a row show significant (p < 0.05) difference among the groups.

The mean serum calcium concentration in the pregnant ewes (clinically sick) cases of hypocalcemia (1.33 ± 0.17 , 1.15 ± 0.31 mmol/l) for the fourth and fifth months of pregnancy, Respectively) was significantly (p < 0.05) lower than the concentration in pregnant ewes (clinically normal) and control (dry ewes) . A significant drop in serum calcium concentration undoubtedly caused clinical hypocalcemia, Excessive loss of calcium in the colostrum and development of the fetus in the late pregnancy, beyond the capacity of absorption from the intestines and mobilization from the bones to replace (Jensen and Swift, 1982; Radostitis *et al*., 2007).

The mean serum magnesium and inorganic phosphorus concentration were also within the normal ranges in fourth and fifth months of gestation comparison with the control ewes, This is in agreement with previous observations shown by different authors (Jensen and Swift, 1982; Tindall , 1986; Sweney and Cudderford, 1987) .

The mean serum glucose concentration (5.65 ± 0.13 mmol/l) in final fifth month of gestation, was found to be significantly (p < 0.05) higher than that of clinically normal ewes (3.83 ± 0.12 and 3.67 ± 0.12 mmol/l in pregnant and non pregnant ewes, respectively). Increased blood glucose concentration was also reported in parturient paretic cows (Radostitis *et al.,* 2007), due to poor tissue glucose utilization. (Schlumbohm, 1997) reported that there is a decreased glucose turnover during late pregnancy in ewes attributed to increase in insulin resistance. It was further indicated that hypocalcemia augments insulin resistance leading to a high serum glucose level. And this is also agree with the (Ganong, 1977) normal secretion of insulin depends on an adequate calcium concentration in the blood and a relationship was seen between low calcium concentration and low insulin concentration in the blood of pregnant ewe, leading to increase glucose concentration in the blood.

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