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Sex preselection of sheep using mineral solution injection via subcutaneous

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ABSTRACT

The injection on Calcium (Ca) and Magnesium (Mg) solution is regarded as a method that have been conducted for sex preselection in sheep. Therefore, this trail was aimed to examine the effect of macro minerals such CaMg subcutaneous injected to Kurdish breed ewes during pre and post mating on lamb sex ratio skewing toward female. Thirty ewes at cycling multiparous period (based on their age and live weight (LW)) were randomly divided into two groups (15 ewes per group). Weekly (for four weeks) from four weeks' days pre- to one week post-mating, each ewe (CaMg group) were subcutaneous injected by 30 ml of CaMg solution or sterile water (control group). Ewes live weigh at the end of the study were numerically higher (2.55 kg) in ewes injected with CaMg compared to control group. The serum ca and Mg were higher ($P<0.05$) in ewes administrated with CaMg solution than those not administrated. The level of total protein, urea, glucose, and cholesterol at blood serum were not affected ($P>0.05$) by CaMg injection. Reproductive hormone such estrogen was not different between control and injected ewes ($P>0.05$). Although, progesterone (at CIDR removal period) were increased ($P<0.05$) in injected ewes than control ewes. The new female born lambs were 2 times higher ($P<0.05$) in CaMg group compared to control group. The results of current study confirm that increasing the mineral proportion (Ca and Mg) in the ewes around mating via subcutaneous injection could skew sex ratio toward female in sheep. Thus, the findings of this trial might assist farmers in managing their breeding toward female lambs (which are going to be born in fattening enterprises and male milk production). More research is required to test different methods of altering mineral concentration and investigate the link between minerals in the diet and human sex pre-selection.

1.Introduction

In animals, offspring sex or gender can mainly be determined through genetic insert obtained from the dams. The allocation of animal sex occurs either by the process throughout each dams that alters the genetic composition of the offspring prior to conception, or as a result of mechanisms that produce sex-biased mortality either during pregnancy or thereafter (Rosenfeld and Roberts, 2004). For these classes, the definition of "primary sex ratio adjustment" refers to the allocation of animal sex mechanisms that begin before to fertilization and cause biases in sex ratio (male and female) offspring. Secondary sex ratio adjustment, on the other hand, applies to allocation of animal sex that occurs following conception, resulting in skewed (offspring gender) male and female survival rates. This might happen during pregnancy or, in circumstances when dams taking care for their offspring, during the period of dams' care (Navara, 2018).

Ruminant animal's requirements for minerals is small (Suttle, 2010). Even though, the animal dry matter intake variation can be significant and it's difficult to make an accurate determinations or quantify of dietary minerals intake (Bicalho et al. 2014; Hussein et al., 2017). Though, NRC (2007) set dietary minerals requirements for ruminant's such sheep, while limitations such as stress, discrepancy in breed, variation in feed intake, and dietary minerals antagonists may result in a reduction in the quantity of nutrient absorption and their bioavailability (Hussein et al., 2017). Consequently, dietary supplementation of minerals through the feed offered to animals may not ensure appropriate mineral intake or availability (Machado et al. 2013). Moreover, mineral intake via drinking water can potentially adversely impact the absorption of minerals from the digestive tract (Spears, 2003; Gould and Kendall, 2011).

Mammals typically deliver approximately equal numbers of offspring (males and females), while there are exceptions to this generality, as found in ruminant animals, where the allocation of their sex theory of Trivers and Willard has delivered a sensible evolutionary underlying to adaptive alterations to the sex proportion (Rosenfeld and Roberts, 2004). Several studies have investigated the influential factors on sex skewing such as the timing of breeding in mice (Krackow and Burgoyne, 1997), modifying the pH of seminal fluid in rabbit (Muehleis and Long, 1976), separation of human albumin sperm (Beernink et al., 1993), and modifying dietary mineral offered to sow (Bolet et al., 1981), rat (Vahidi and Sheikhha, 2007), chicken (Saleh and Iriyanti, 2011), dairy cattle (Stolkowski and Lorrain, 1982), or sheep (Alhimaidi et al., 2021). Nevertheless, more studies were recommending to conduct using animal module in order to elucidate the link between altering dietary minerals with respect to human sex pre-selection (Alhimaidi et al., 2021; Gharibi et al., 2023).

In Iraq, sheep play a vital role in socioeconomic and livelihood of the farmers particularly in rural are by supplying products such as wool, meat, and diary. Besides, sheep have been employed in genetic modification studies. Hence, they were employed in the current trial to investigate offspring sex preselection. Therefore, subcutaneous injection of minerals such Ca and Mg could potentially offer an alternative method of modification in dietary mineral. To the best our knowledge, the impact of a subcutaneous administration of solution containing CaMg around mating of ewe to manipulate skewing in sex ratio has not previously investigated. Therefore, this study was sought to test the impact of subcutaneous mineral injection to skew sex ratio of new born offspring toward female in Kurdish ewe.

2.MATERIALS AND METHODS

2.1. Animal Ethic Protocol

Scientific committee animal resources department of the Salahaddin University-Erbil approved the ethic protocol for the current study.

2.2. Experimental animal location and Design

A study was conducted at one of the private farm in Daratw District in Erbil City during the period from June to October 2023 using 30 Kurdi breed ewes (initial live body weight) of 58.92± 2.97 kg for five months. Ewes were assigned randomly (based on their live body weight) to one of two treatment goups (control and CaMg groups), with 15 ewes per group. The ewes were kept in a well-ventilated shed in groups. Ewes throughout the

trial had free accessed to water.

2.3. Estrous synchronization protocol and pregnancy diagnosis

The synchronisation were done to synchronise estrous cycle of all experimental ewes (after sixteen days of the lunching of flush feeding and injection of CaMg solution) by using CIDRs (Eazi-Breed CIDR® Sheep and Goat Inseet, New Zealand). The CIDRs were placed in ewes intra-vaginal for a period of 10 days. One day before CIDR removal, the volume 2 ml of D-Cloprostenol (prostaglandin hormone, Vetaglandin®, Abu Raihan Co, Tehran, Iran) were intramuscular IM injected for each ewes. This procedure was to get rid of any previous pregnancy experience, or to remove polycystic ovaries or the presence of corpus luteum. At the same time, ewes were injected via IM with 600 IU of PMSG (eCG) hormone (SYNCRO-PART®, Ceva Sante Animal, France). Moreover, a week before CIDR removal and on the day of CIDR removal, ewes also subcutaneously received 2 mL a mixture solution contained vitamin A D3, and E; 3VitADE, Fariman, Iran). Additionally, by the same method, a volume of 2.5 mL of a solution contained vitamin E and mineral Selenium (Selevet, Belekotechnika Co, Republic of Belarus) at the day of CIDR removal were injected. The mating procedure were performed by Kurdish rams breed for all ewes (for 3 days) at a ratio of 7 ram for 30 ewes. Pregnancies were identified 30 days' post mating via ultrasound (Prosound 2, Aloka, Japan).

2.4. Diets

The diet was similar for both control and CaMg groups. Ewes were offered flushing diet (concentrate with forage-wheat straw). Both concentrate and straw were manually mixed during offering to animals with unknown ratio (which was conventionally practiced by farmers). The concentrate diet was a commercial dried pelleted (Erbil Feed, for livestock concentrate feed manufacture, Erbil, Iraq), including wheat bran (32%), whole barley (25%), whole wheat (16.5%), corn grain (14.0%), soybean meal (4%), soy oil (1%), molasses (7%). The approximated analysis for feed samples (straw and concentrate) were conducted at commercial feed laboratory (Erbil feed laboratory for chemical analysis for livestock feed, Erbil, Iraq) for DM (91.3,90.3 g/kg), Ash (58,71), CP (29,114), EE (13,43), and crude fiber (44,38) contents (g/kg DM) respectively.

2.5. Treatments

Ewes were allocated by live weigh to one of two groups (control and CaMg groups). The control group were subcutaneously administrated with 30 ml of sterile water. While, the same volume of CaMg solution (Calcium inject, HEBEI KEXING CO, China) were administrated for CaMg group. Each 100 mL of CaMg solution contained 32.83 g of Ca gluconate, 8.13 g of Ca glecerophosphate, and 4.18 g of Mg chloride. There injections were every 7 days 28 days pre-mating and 7 days post-mating.

2.6. Live Body Weight Determination

Ewes were weighed twice. The first, at the beginning of the study, and the second at day of CIDR removal. The weighing was conducted by using digital weigh scale, and the scale was calibrated prior to use and every 6 weighing using standard weights for precision.

2.7. Blood Sample Collection and Analysis

Blood collection was performed via jugular venipuncture using a 20 gauge 1.5" needle (Becton Dickinson Vacutainer Systems, Plymouth, UK) on the three different periods (commencement of the study, day of removal CIDRs, day of mating, and a month days after mating) at 12:00h. The silica tubes were used to collect blood samples that were kept overnight in a refrigerator to coagulate. Then, all tubes were centrifuged at 1000 g for 15 min at a 4 ºC using a (Beckman Avanti 30). Following that, harvesting serum was then transferred into 2 ml bijou tubes and stored at – 20 ºC for subsequent serum Ca, Mg, total protein, total cholesterol, glucose, estradiol, and progesterone analysis.

Blood serum metabolite parameters such as cholesterol and glucose were analysis at MIDLINE laboratory (commercial laboratory, Erbil, Iraq) as described by (Qoja and Hussein, 2021). Blood serum both Ca and Mg were demined by Piccolo Auto analyzer (PICCOLO CHEM ANALYZER, Gesan®-Italy). Serum estradiol and progesterone were determined via ELISA reader (STAT-FAX 3200, USA).

2.8. Statistical Analysis

The MIXED procedure of SAS (2003) was employed to analyze data for body live weight, hormones and blood metabolites. The reproductive performance parameters were statistically calculated as described by Gharibi et al. (2023). The reproductive parameters (%) were analyzed using the chi-square test. Significance differences between control and CaMg groups were determined at $P < 0.05$ level. The differences were compared using protected least significant difference test.

3.RESULT AND DISCUSSIONS

3.1. Effect of CaMg injection on ewe body weight

Around mating, there was no effect ($P>0.05$) of CaMg-injected ewes' live body weight (Fig. 1). However, at CIDR removal day, ewes live weight in injected group (56.43 kg) were numerically lower compared to control group (58.98, s.e.d; 2.926). Previously, the injection of CaMg tended to reduce ewes body weight (Gharebi et al., 2023). In contrast, dietary supplementation of Ca and Mg didn't affect ewes body weight (Alhimaidi et al., 2021). The mechanism by which macro mineral such Ca influencing adiposity could be attributable to its level in intracellular and its influence on both lipogenesis and lipolysis process in adipocytes. Thus, decreased Ca intake leads in reduce serum ionized Ca. While, boosting PTH production that enhance Ca influx into adipocytes. The elevation of intracellular Ca levels in adipocytes promotes lipogenesis and inhibits lipolysis, leading to the preservation of fat stores. Conversely, an increase in dietary Ca intake, which leads to increased levels of ionized Ca in the blood, inhibits the release of parathyroid hormone (PTH) and promotes lipolysis. Furthermore, Mg may strengthen the positive effects of Ca in reducing body fat (Seki et al., 2013). This evidence clearly suggests that changes in body weight around the female have a significant impact on sex ratios, rather than the female herself possessing an innate feature. Consequently, conducting research that examine alteration in animal sex ratio, relating to factors that are known to affect dams body condition score is critical for understanding the mystery of sex ratios.

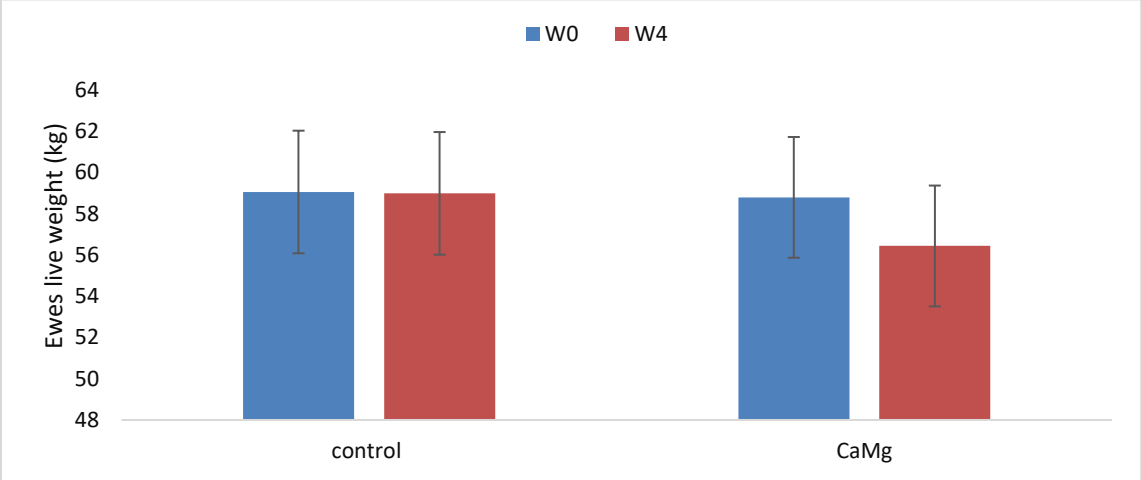

Figure 1. the effect of CaMg subcutaneous injection on ewes live weight (kg)

3.2.Serum Ca and Mg levels of ewes

The ewes serum concentration of both Ca and Mg was increased ($P<0.05$) after subcutaneous injection of CaMg in comparison with non-injected ewes (Table 1). Similarly, both Alhimaidi et al (2021) and Garibi et al (2023) reported the increase of Ca and Mg after administration of CaMg in ewes. Recently, dietary supplementation of CaMg of rabbit resulted in significant increase in serum Ca and Mg (Naidu et al., 2023).

Table 1. Serum Calcium and Magnesium concentration of ewes throughout study period[1]

Parameters	Control	CaMg
Calcium, mg/dL		
Start of trial	8.59±0.27	8.72±0.14
CIDR removal day	9.17±0.16[b]	9.52±0.15[a]
Estrous and mating period	8.55±0.30[b]	9.45±0.14[a]
Magnesium, mg/dL		
Start of trial	3.19±0.05	3.20±0.11
CIDR removal day	3.13±0.03	3.54±0.05
Estrous and mating period	3.08± 0.01[b]	3.39±0.08[a]

[1]Means od serum Ca and Mg in the same row with different letters are different significantly at p<0.05.

3.3. Admiration of CaMg effects on blood serum total protein, glucose, and cholesterol levels

Blood serum concentration of total protein, urea, glucose, and cholesterol were within normal range (Abdulkareem et al., 2020; Qoja and Hussein, 2022) (Table 2). The administration of CaMg solution had no effect (P>0.05) on blood serum biochemical parameters throughout the study period. This result is in lined with Gharebi et al. (2023) who also reported no effect of admiration of CaMg solution in KurdishxRomanov ewes.

Table 2. Blood serum biochemical parameters of ewes subcutaneously injected or non-injected with CaMg solution[1]

Parameters	Control	CaMG
Total Protein, mg/dL		
Start of trial	5.01±0.17[a]	5.21±0.19[a]
CIDR removal day	6.86±0.14[a]	7.30±0.21[a]
Estrous and mating period	7.26±0.11[a]	7.28±0.17[a]
Urea, mg/dL		
Start of trial	14.16±0.13[a]	14.12±0.12[a]
CIDR removal day	15.24±0.35[a]	15.01±0.16[a]
Estrous and mating period	15.61±0.31[a]	16.82±0.16[a]
Glucose, mg/dL		
Start of trial	68.39±2.81[a]	73.87±1.64[a]
CIDR removal day	62.87±2.98[a]	69.90±1.62[a]
Estrous and mating period	68.05±2.70[a]	72.63±1.67[a]
Cholesterol, mg/dL		
Start of trial	55.97±2.26[a]	60.65±2.56[a]
CIDR removal day	53.71±2.39[a]	57.36±2.42[a]
Estrous and mating period	58.70±2.49[a]	59.25±2.33[a]

[1]Means of blood serum biochemical parameters in each rows with the superscript (a) were not significantly different.

3.4. Effect of CaMg injection on reproductive hormones levels

There is a role of reproductive hormones on skewing the sex ratio, and affecting both males and females (Navara, 2018). It has been hypothesized that increased levels of testosterone in both male and female dams contribute to increase new born male offspring. Conversely, high levels of progesterone resulted in decrease male offspring (James, 2008). In this study, CaMg injection increased both progesterone (significantly; P>0.05) and estrogen (numerically) at CIDR removal day) compared to non-injected

ewes (Table 3). The higher level of reproductive hormone such progesterone has been linked with sex ratio skewing toward female (Navara, 2018).

Table 3. Serum hormones level of ewes subcutaneously injected or non-injected with CaMg solution

Estrogen, pg/mL		
Start of trial	61.58±1.72	68.53±0/17
CIDR removal day	63.27±1.68	70.11±0.98
Estrous and mating period	74.03±0.92	79.70±0.95
Progesterone, ng/mL		
Start of trial	2.01±0.04	1.90±0.03
CIDR removal day	1.88±0.01b	1.99±0.04a
Estrous and mating period	0.69±0.02	0.92±0.01

[1]Different superscripts in the same row differ significantly (P < 0.05).

3.5.The effect of maternal injection with CaMg on the sex ratio of newborn lambs

The effects of administration of both macromeres (CaMg) on skewing sex ratio in lambs were previously examined in a research conducted Gharibi et al. (2023). According to their results, ewes who supplemented with Ca and Mg produced higher percentage of female lambs. Similarly, in rat (Arangasamy et al., 2015) and rabbit (Naidu et al., 2023). The addition of CaMg resulted in engendered greater female. The results of current study were in accordance with above findings where CaMg injected group had significantly (P<0.05) higher female offspring compared to non-injected group (Table 4). It is not well understood which underlying mechanism that CaMg employ to impact the sex ratio in animals their offspring. Nonetheless, it has been proposed that CaMg may affect both function and synthesis and of reproduction hormones (progesterone and estrogen) that are important for embryonic development. As evidence for this, a research by Hassan et al. (2022) shown that sex-specific morphological alterations and mineral deposit in female offspring were caused by altering the Ca:P ratio in the dams diet.

In current study the level of both serum progesterone and estrogen in injected group were higher compared to control group. Similarly, Gharibi et al. (2022) found higher levels of serum progesterone and estrogen when ewes were injected by Ca and Mg of which female offspring in injected group were correspondingly higher compared to control group. Thus, it can be state that Ca and Mg subcutaneously injected may result in increased (progesterone and estrogen) female reproductive hormones and which in turn hormones changed sex ratio. Different doses (0.25 mg and 2 mg) of progesterone in laying hens were tested for their effects on the sex ratio following injection (Correa et al., 2005). They reported that the high dose (2mg) of progesterone resulted in a significant decrease in the ratio of male offspring in those eggs, with 25% of males produced in the 2mg progesterone group and 61% and 63% in the other two groups (control and 0.25mg). The higher female or low male was attributed to the segregating of sex chromosomes, and these chickens' progesterone levels were noticeably higher. This shows that variations in progesterone concentrations can impact sex proportions; however, due to progesterone is a precursor to other sex steroids such testosterone. Though, it is unclear whether progesterone acted indirectly or directly through testosterone's downstream effects.

Table 4. Reproductive parameters of ewes injected with or without CaMg during mating[1]

Treatments	No. of ewes	No. of pregnant ewes (Ratio%)	No. of new born lambs	No. of male lambs	No. of female lambs
Control	15	15 (100%)	14	9 (64.29%)[a]	5 (35.71%)[b]
CaMg	15	15 (100%)	15	5 (33.33%)[b]	10 (66.67%)[a]

[1]Means with different superscripts in the same column or row differ significantly ($P < 0.05$).

4.CONCLUSION

Ewes Subcutaneously injected with (a macro minerals Ca and Mg) solution around mating resulted in altering sex ratio towards female. Which was consistence with the high serum progesterone and estrogen levels. Moreover, the levels of both serum Ca and Mg were correspondingly increased by the injection. Ewes weigh at the end of the study were numerically lower when CaMg solution were administrated. The mechanism behind sex ratio skewing in animals is still unknown. The current study's findings, however, suggest that altering serum Ca and Mg concentrations around mating might be used to skew the sex ratio toward females in sheep. In addition, it could be recommending that injection CaMg showed safe, economy, and comfortable method to apply without have a notable and extreme side consequence on the animal's health and production.

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