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Original article

Sex preselection of sheep embryo by altering the minerals of maternal nutrition

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ABSTRACT

Several methods have been conducted for embryo sex preselection, which includes X- and Y- sperm separation, changing the pH of the female reproductive tract, time of mating before or after ovulation, and feeding formula, such as altering the presence of minerals in diet content before breeding may affect the embryo sex preselection ratio. In this study, three food formulas to feed female sheep were created with the cooperation of the Arabian Agricultural Services Company (Arasco). Ewes were fed with modified food formulas for one month before mating with males. The first group (A) (30 ewes), modified for male embryo gender preselection, were fed a diet with an increased percentage of the minerals Na⁺, K⁺ and P⁻. The second group (B) (30 ewes), modified for female sex preselection, were fed a diet with an increased percentage of the minerals Ca⁺⁺ and Mg⁺⁺. The third (control) group (C) (30 ewes) were fed the regular (Wafi) food formula. Our results showed no significant differences were in mean body weights between the three groups at the end of the feeding period. The results of different feeding formulas on mineral serum blood samples of ewes showed an increase in Na⁺, K⁺ and Cl⁻ ions in the serum of group (A) compared to the other groups (B and C). The concentration of Na⁺ in the serum of group (A) was significantly ($P < 0.05$) higher than group (C). The concentration of Cl⁻ ions in serum samples of ewes in group (A) was significantly higher than group (C) and group (B) ($P < 0.05$). The role of maternal feeding on embryo sex preselection shows that the pregnancy rate of animals in group (A) was 73.33%. Group (A) birthed 17 males and 5 females (77.27% and 22.72%, respectively). The pregnancy rate in group (B) was 70%. Group (B) birthed 6 males (27.27%) and 16 females (72.72%). Finally, the control group (C) had a pregnancy rate of 76.66%. They birthed 13 males (54.41%) and 11 females (44.83%). The results of our study confirm that altering the percentage of minerals in the maternal diet plays a role in sex preselection in sheep, which agree with other mammalian studies in rats and mice. Thus, the result of this study can help farmers to manage their breeding. We recommend that more studies on the relationship between minerals in the diet should be conducted for other species and human sex preselection.

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1. Introduction

The preselection of fetal sex before pregnancy has received considerable attention and controversy over the years. Several methods have been conducted with recent technological developments in order to provide up to 80% of couples willing and interested in selecting their child's sex according to their wishes (Hewitt, 1987). Several experiments have investigated the factors that influence the rate of sex constitution, (Cramer and Lumey, 2010; Çelik et al., 2003). Such as the timing of mating,

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might have a role in the sex ratio in mice (Krackow and Burgoyne, 1997). The altering the pH of seminal fluid may change the sex ratio of rabbit offspring (Muehleis and Long, 1976). The accomplishment of sex preselection were done via albumin separation of sperm (Beernink et al., 1993). The sexing of mammalian sperm can be used for the production of offspring (Johnson, 2000). The influence of the mineral diet of sows affect the sex ratio of their newborns (Bolet et al., 1981). Experiments on pre- and post-conception mechanisms demonstrated that these mechanisms influence the sex ratio in mouse embryos (Nez et al., 2003). Some studies based on theories that sperm carrying the X or Y chromosome favored different vaginal pH. Therefore, it has been suggested that the pH of the vagina at the time of fertilization may have a differential effect on X- or Y- sperm, thus affecting the sex of the offspring (Muehleis and Long, 1976). Other investigations have shown that higher concentrations of K^+ and Na^+ and lower concentrations of Mg^{2+} and Ca^{2+} in a diet of sows (Bolet et al., 1981), for dairy cattle (Stolkowski and Lorrain, 1982), and rats (Vahidi and Sheikhha, 2007) increased the sex ratio of males to females, in mice (Alfageeh and Alhimaiddi, 2013), and in chicken (Saleh and Iriyanti, 2011). It has been found that feeding mineral nutrients to animals may especially modify the secretion of the exocrine glands in the oviduct. This modification may provide an environment that likely favors one of two sperm (X or Y) to live or race to fertilize the ovum in the oviduct. Additionally, the change in nutrients might modify or change in the composition of the sperm receptors on the surface of the oocytes (zona pellucida) that may have a role in the sex preselection concerning the association with the X or Y sperm (Alfageeh and Alhimaiddi, 2013).

Sheep play an important role as a supplier of meat, milk, and wool. Sheep are also commonly used in genetic modification experiments. Thus, they were used in the present study to explore fetal sex preselection. This study aims to produce two types of food formula (one for producing male fetuses and the other for producing female ones) to investigate the influence of maternal nutrition during the pre-mating period on sheep fetal sex preselection. We believe that the increase in certain minerals concentration in the maternal diet might affect the sex of the fetus.

2. Materials and methods

All animal procedures and treatments were conducted according to ethics committee guidelines at the College of Science, Zoology Dept. at King Saud University, Riyadh Saudi Arabia.

Animal body weight and number: Ewes in all groups were given numbers using ear tags. The ewes were weighted before and at the end of the experimental feeding period (one month). Two blood samples were collected from each ewe: at the start of the experiment (day 1) and the end of the experimental feeding period (day 30). The blood samples were used to estimate the macromineral levels of the ewes; these minerals included calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), and chloride (Cl). The blood samples were collected from the jugular vein into 5 ml serum collection tubes (BD, USA) without anticoagulant using a 5 ml syringe (Luer-Lok tip, BD, USA) attached to 18 G \times 1 1/2 in. needle. After collection, the samples were transferred to our laboratory using an ice box. Then, they were centrifuged at 3000 rpm for 10 min to separate the serum (Universal 320, Hettich, Germany). The serum was transferred into 1.5 ml Eppendorf tubes and kept at -80°C until use. The macromineral level was measured using a semi-automatic biochemistry analyzer (Udichemplus, Spain). Serum samples were removed from -80°C and kept at room temperature until they defrosted. Then, six macromineral include sodium, calcium, potassium, inorganic phosphorus, mag-

nesium, and chloride, were examined using semi-automated clinical chemistry kits (UDI, Dammam, KSA).

3. Food formula

The three types of food formulas used in this study designed in cooperation with and donated from the Arabian Agricultural Services Company (Arasco). The composition of each formula were illustrated in Table 1. The first food formula designed for male sex preselection (group A) has an increased concentration in the following minerals (compared to the control (C) (Wafi) group food formula): sodium (Na^+), potassium (K^+), and phosphorus (P^-) ions. The second food formula designed for female sex preselection (group B) has an increased concentration in the following minerals (compared to the control (C) (Wafi) group food formula): calcium (Ca^{+2}) and magnesium (Mg^{+2}) ions. The third food formula, the standard food formula (Wafi), given to be the control group (C) Table 1.

4. Sheep maternal feeding for one month

The three groups (A, B, and C; 30 ewes per group) were fed for one month. Then, 40 mg sheep sponge, progesterone impregnated intra-vaginal sponge (from SYNCRO-PART[®], Ceva Sante Animal, France), was inserted into each ewe 16 days after the start of the experiment for a period of 14 days. At the same time, each ewe was injected with 2 ml of prostaglandin hormone [Estrumate synthetic prostaglandin, Intervet International B.V., European Union (EU)], via intramuscular injection (IM) to get rid of any previous pregnancy experience, or to remove polycystic ovaries or the presence of corpus luteum. On the day of sponge removal, all ewes were injected via IM with 600 IU of PMSG (eCG) hormone (SYNCRO-PART[®], Ceva Sante Animal, France). Later, rams were randomly introduced into the three groups 36 h after sponge removal for natural mating. Pregnancies were detected approximately 25–30 days from the day of mating using an ultrasound (Prosound 2, Aloka, Japan) (Anwar et al., 2008).

4.1. Statistical analysis

Data were collected and analyzed by analysis of variance using a GLM procedure as a completely randomized design with repeated measurements. The differences between the mean were tested with a protected least significant difference test (S.A.S., 1982).

5. Results

5.1. Effect of different feeding formula on ewe body weight

The body weights of all female sheep were recorded at the beginning and end of the first month of feeding. The average weights of ewes after one month were (55.70 ± 1.67) in the male sex preselection group (A), (57.15 ± 2.55) in the female sex preselection group (food formula B), and (55.30 ± 3.90) in the control group (C). The average weights at the beginning of the study were (59.85 ± 1.85), (60.75 ± 2.93), and (55.30 ± 4.22) in A, B, and C, respectively, as shown in Table 2.

These results showed no significant differences in mean body weights between the three groups at the end of the feeding period. There was a significant ($P < 0.05$) decrease in the average body weight of sheep at the end of the experiment in groups A and B (-4.15 and -3.6 , respectively) compared to the body weight on day one.

Table 1

The three types of food formula A: Male sex preselection; B: female sex preselection; and C: Control regular food formula.

Notes	C: Control	B: Female preselection	A: Male preselection	Food component
	18.00%	18.00%	18.00%	Protein
	3.00%	5.50%	3.00%	Crude Fat
	6.00%	8.00%	6.00%	Crude Fiber
	6.50%	6.50%	6.50%	Ash
Male	0.70%	0.50%	0.90%	Salt (Na⁺ Cl⁻)
Male	0.60%	0.50%	0.70%	Phosphorous (P)
Male	1.00%	0.80%	1.2%	Potassium (K⁺)
Female	1.00%	1.20%	0.80%	Calcium (Ca ⁺⁺)
Female	0.32%	0.35%	0.30%	Magnesium (Mg ⁺⁺)
Female	3 IU/g	4 IU/g	2 IU/g	Vitamin -D
	20 IU/g	20 IU/g	20 IU/g	Vitamin -A
	15 IU/kg	15 IU/kg	15 IU/kg	Vitamin E
	2780 kcl/kg	2780 kcl/kg	2780 kcl/kg	Energy (ME kcl/kg)
Trace Minerals added (>0.1) Cobalt, Copper, Iodine, Selenium, and Zinc. IU = International Units.				

Table 2

Mean body weight of ewes through the experiment (Mean ± SE).

Parameters	Groups		
	Group A	Group B	Group C
BW (kg)			
Day 1 of feeding	(59.85 ± 1.85) ^a	(60.75 ± 2.93) ^a	(55.30 ± 4.22) ^a
Day 30 of feeding	(55.70 ± 1.67) ^a	(57.15 ± 2.55) ^a	(55.30 ± 3.90) ^a

Means in each rows with the superscript (a) were not significantly different. Group (A) is the male sex preselection, group (B) is the female sex preselection, and group (C) is the control.

6. Effect of different feeding formula on mineral serum blood samples of ewes:

An increase in Na⁺, K⁺ and Cl⁻ ions in serum samples of females was observed in the group (A) compared to the other groups (B and C). The concentration of Na⁺ ion in serum samples of females in group (A) was significantly higher than in group (C) (P < 0.05), and non-significantly higher than group B. The concentration of Cl⁻ ion in the serum samples of females in group (A) was significantly higher than group (C) and group (B) (P < 0.05). The serum concentration of Mg⁺², Ca⁺², and P⁻ ions in females in group (B) was non-significant higher than females in other groups (A) and (C) [Table 3](#).

7. The role of maternal feeding on embryo sex preselection

Our results show that for the animals in group (A), which were under a high sodium, potassium, and chloride diet, 22 out of 30 ewes (73.33%) became pregnant and delivered 22 offspring. The subsequent offspring were 17 males and 5 females (77.27% male and 22.72% female). In group (B), under a high calcium and magnesium diet, 21 out of 30 ewes (70%) became pregnant and delivered 22 offspring (one of the ewes delivered twins). The subsequent offspring were 6 males (27.27%) and 16 females (72.72%). Finally, in the control group (C) 23 out of 30 ewes became pregnant (76.66%; one ewe delivered twins). The subsequent offspring were 13 males (54.41%) and 11 females (44.83%) [Table 4](#).

Table 3

The concentrations of six minerals in serum samples taken from ewes in different group average before and after the experimental feeding period (Mean ± SE).

Groups	Na ⁺ (mEq/l)	K ⁺ (mEq/l)	Mg ⁺² (mg/dl)	Ca ⁺² (mg/dl)	Cl ⁻ (mEq/l)	P ⁻ (mg/dl)
C	137.29 ± 3.56 ^b	9.58 ± 0.14 ^a	2.53 ± 0.11 ^a	9.53 ± 0.39 ^a	135.43 ± 4.95 ^b	6.38 ± 0.23 ^a
A	157.26 ± 3.63 ^a	9.95 ± 0.35 ^a	2.29 ± 0.09 ^a	10.27 ± 0.30 ^a	174.54 ± 11.70 ^a	6.21 ± 0.20 ^a
B	155.83 ± 5.29 ^a	9.47 ± 0.39 ^a	2.54 ± 0.08 ^a	10.49 ± 0.34 ^a	142.08 ± 4.84 ^b	6.77 ± 0.18 ^a

Different superscripts (a and b) in the same column differ significantly (P < 0.05). (A) Male sex preselection, (B) female sex preselection, and (C) control group.

8. Discussion paraphrase

Several previous studies ([Hewitt, 1987](#); [Bolet et al., 1981](#)) do not appear to be sufficient enough to explain the relationships between sex constitution and minerals consumed. In this study, the mineral levels and sex ratios obtained from ewes consuming different mineral diets were compared. Our results in [Table 2](#) show a non-significant decrease in body weight in ewes of treated groups compared to the control group. We believe that this non-significant decrease is due to the poor palatability of the experimental feeding formula, which contains different concentrations of target minerals compared to the regular one. This result is consistent with ([Green et al., 2008](#)), who found that the addition of polyunsaturated fatty acid (PUFA) to the ewes' diet did not affect the body weight and ewe weights remained relatively constant over the five months of the study. [Table 3](#) represents the different 8mineral levels in ewe serum in all groups. The Na, K, and Cl ions levels in the serum of ewes in group(A), at the same time the Mg, Ca, and P ions levels in the serum of ewes in group(B)(increase related to increase the amount of these minerals in group (A) and group (B) feeding formula respectively, compared to control group (C). A study conducted on the serum electrolytes concentration in Awassi sheep ([AL-Hadithy et al., 2012](#)). They found that the range and mean ± standard error (SE) were as follows: serum sodium 101.10–191.20 mmol/l and 155.43 ± 1.46 mmol/l, serum potassium 3.20–15.10 mmol/l and 7.33 ± 0.23 mmol/l, and serum chloride 70.40–184.20 mmol/l and 110.85 ± 1.85 mmol/l. They found a significant difference (P < 0.05) between males and females in those serum ions levels. However, there was no significance between male groups and female lambs nor among different female subgroups (except significantly higher in the sodium concentration recorded in the first gestation, P < 0.05). So the serum levels of (Na, K, and Cl) were significantly higher in males compared to females with significant differences according to the physiologic status of the female groups ([AL-Hadithy et al., 2012](#)). The whole measurement of blood and serum mineral concentrations of diverse bighorn sheep (*Ovis canadensis*) in California, they found that there were some statistical differences between the median

Table 4
Sex ratio and sex preselection depending on the sheep diet groups.

Groups	No. of females	No. of pregnant females	Pregnancy rate %	Number of offspring	No. of male offspring (Ratio %)	No. of female offspring (Ratio %)
C	30	23/30	76.66%	24	13 (54.17%)	11 (44.83%)
A	30	22/30	73.33%	22	17 (77.27%) ^a	5 (22.73%) ^b
B	30	21/30	70%	22	6 (27.27%) ^b	16 (72.73%) ^a

Different superscripts (a and b) in the same column or row differ significantly ($P < 0.005$).
(A) Male sex preselection, (B) female sex preselection, and (C) control group.

concentrations among the different metapopulations (Poppenga et al., 2012). The detected values were generally in good agreement with concentrations reported for other bighorn sheep populations and with reference ranges widely accepted for domestic sheep (*Ovis aries*). Thus, there are several factors that can influence mineral concentrations in wildlife species, such as bighorn sheep (Poppenga et al., 2012). Regarding the effect of the maternal diet on sex preselection, our results show that modifying the minerals in the food formula skews the offspring sex and affects the sex ratio. The results of our studies confirm that altering the percent of minerals in the maternal diet has a role in sex preselection, which agrees with other mammalian review studies (Meikle and Thornton, 1995), such as (Vahidi and Sheikhha, 2007; Chandrāju et al., 2013) in rats; (Alfageeh and Alhimaidi, 2013; Noorlander et al., 2010) in mice. In sheep, was found that changing the amount of polyunsaturated fatty acid in the maternal diet also affects sex preselection (Green et al., 2008). Moreover, found that the combination of maternal diet and the timing of intercourse can increase the probability of conceiving a girl by 81% in humans (Rosenfeld and Roberts, 2004). Several hypotheses may explain the outcome from the addition of minerals into mammals' diet. The first hypothesis is related to spermatozoon; the sperm of one sex might have differential motility or make their way more directly to the oocyte than the other depending on the conditions prevailing in the reproductive tract of the impregnated female (e.g., state of cervical mucus, nutrient/energy status of tract secretions, vaginal pH relative to the precise time at which copulation occurred concerning estrus) (Pratt et al., 1987; Martin, 1997). One class of sperm might have intrinsic physiological differences in viability, capacitation, or acrosome reaction dynamics (Madrid-Bury et al., 2003; Gutierrez-Adan et al., 1999). The other hypothesis is that the sperm of one sex might be more capable of influencing fertilization once the egg has been reached depending on factors such as the condition of the female reproductive tract and the penetrability of the zona pellucida, which likely vary according to the time of ovulation relative to the time of insemination. Depending on the maturational state at the time of fertilization, the oocyte might preferentially bind to X- or Y-bearing sperm (Dominko and First, 1997). The last hypothesis is that the sperm receptors on oocytes zona pellucida depend on the conditions prevailing in the reproductive tract of the female. The oocyte may change the receptor structure or function to one sex sperm or synthesis on receptors specific to one of the sex sperm. So we recommend altering the mineral percentage in the diets of animals, which is cheap, easy, and safe to use with no extreme side effects to the animals. The results of this study also help farmers manage their breeding. We recommend that more studies on the relationship between changing minerals in the diet should be conducted with regard to human sex preselection.

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Declaration of Competing Interest

The authors declare no competing or financial interests.

Ethics approval

All of the experiments were conducted according to the Guidelines for the Institutional Animal Care and Use Committee of the Zoology Department, College of Sci. at King Saud University.

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