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Experiments to Investigate the Factors That Affect the Rate of Sex Constitution

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Abstract: This paper reports a study on mice and human to check the theory of Stolkowski which hypothesizes that mineral (Na, K, Ca) imbalance in the diet of the female before fertilization affects the sex ratio of the progeny. For this aim, total 40 female and 16 male 2 months age white mice used; which were separated into 8 groups, each of them consisted of 5 female and 2 male animals. Group 1 was a control, group 2-3-4-5-6-7 and 8 were treated as experimental groups. (0.5 g Na/kg feed, 2,0 g K/kg feed and 5,0 g Ca/kg feed); feed was given to group 1 while group 2-3-4-5-6-7 and 8 received in their feed 1.5 g Na/kg feed, 6,0 g K/kg feed and 15,0 g Ca/kg feed during 30 days before mating. K had the highest male ratio in their litter followed by those given Ca+K. Population of Çanakkale was evaluated by traditional nutrition of habits and no found statistical differences in sex both m/f (P>0.10).

Key words: Minerals, feed, sex, mice, human, Çanakkale

Introduction

Predetermination of sex in human and in farm animals is reviewed. Preconceptional sex selection has generated great interest and controversy over the years. Medical and commercial benefits outweigh the ethical issues. Technology has not yet provided a routine method for separating the X- and Y-chromosome-bearing sperm. Predetermination of sex in livestock offspring is in great demand and is of critical importance to providing for the most efficient production of the world's food supply. With the changes that have taken place in animal agriculture over the past generation the application of sex preselection to production systems becomes increasingly necessary (Johnson, 2000). The desire to control the sex of our offspring prior to conception has been present throughout man's and livestock's history. In recent years the application of a more scientific approach to the problem has resulted in techniques capable of providing interested couples with an 80% chance of having either a boy or a girl according to their wishes in human (Hewitt, 1998).

Scientists researching cellular communication have found that the mother's ovum membrane's receptors had an alternating polarity (energy) which accepts or rejects the "x" or "y" chromosome sperm according to her energy cycle. Previous research had shown that the "y" chromosome sperm had a constant different charge to "x" chromosome sperm. This was nature's way of varying the gender of humans and other mammals. The charge on the ovum membrane was not fixed but alternated from positive to neutral and to a negative charge in a cycle. This was called the polarity cycle of the ovum membrane. This polarity was found to be predictable but totally separate from the menstrual cycle. The polarity cycle which unknown to most of us, was there in addition to the ovulation/menstrual cycle. When the ovum membrane is neutral, sperms carrying either the X or Y chromosome can unite with the ovum and fertilize it. During this period, the chances of getting a baby boy or baby girl are 50 : 50. When the ovum membrane is positively charged, it attracts the sperm carrying X chromosome (which is negatively charged) and a baby girl is produced. Today one of good known methods on sex constitution, is the preconception diet method. This method claims 80% accuracy and the theory is that by altering your diet to include and exclude certain foods, the condition in the reproductive tract will be directly affected, increasing the odds of conceiving a particular sex It is also recommended that both mother and father go on the diet. This theory is also consistent with the oriental philosophy that everything has a yin or yang quality and the foods supplied in the boy diet, boys, and alkaline are all yang and the foods supplied in the girl diet, girls and acid are all yin. The girl diet is high in calcium but low in salt and potassium, containing acid forming foods. The boy diet is high in salt and potassium but low in calcium and magnesium and contains alkali-forming foods. The diets nutritional content is questionable and contains multiple warnings. The diet may influence the conditions within the reproductive tract and the outer barrier surrounding the ovum. Enabling only one of the two types of sperm to penetrate the egg depending on which diet is adhered to. Langendoen and Proctor first published 'The Pre-Conception Gender Diet' based on results reported by Stolkowski and Lorrain (1982). The theory is that by altering your diet to include and exclude certain foods, the conditions in the reproductive tract will be directly

affected, increasing the odds of conceiving a particular sex. This method under scrutiny claims of 80% accuracy based on one clinical trial of only 260 women, the results were published in the International Journal of Gynecology and Obstetrics in 1980. The girl diet is high in calcium but low in salt and potassium, containing acid forming foods. The boy diet is high in salt and potassium but low in sodium, calcium, magnesium and contains alkali-forming foods. The diets nutritional content is questionable and contains multiple warnings. It is recommended that you seek the advise of you medical practitioner before going on such a restrictive diet, and stay on the diet for no longer than 3 months. The diet may influence the condition of the cervical mucus and within the reproductive tract and follicular fluid. Enabling only one of the two types of sperm to penetrate the egg depending on which diet is adhered to. The aim of this study was to evaluate relationship between minerals and sex ratio in mice and human.

Materials and Methods

Mice and diets: Female (40) and male (8) 2 months age mice were obtained from Çukurova University Dept. of Experimental Surgery Center (TIPDAM) and transferred to community cages situated in a open shed and temperature-controlled (20 ^{o}C) room. Treatments were divided into Control, (C), K, Ca, Na, Ca+K, Na+K, Ca+Na, and Na+K+Ca groups. Each treatments were given 2 male for mating. Declareted minerals was 0.5 g Na/kg feed, 2,0 g K/kg feed and 5,0 g Ca/kg feed which provide guidelines for the adequate mineral nutrition of mice maintained in conventional animal facilities Table 1. Diets were prepared 1.5 g Na/kg feed, 6,0 g K/kg feed and 15,0 g Ca/kg feed and water were given *ad libitum*.

Statistical Analyses: Ratio of male sibs in a litter was calculated by dividing the number of males in the litter to the total number of animals. Repeated measures model was fit for analyses using the repeated statement in proc mixed procedure of SAS V8 (SAS Institute Inc., 1999). The model included type of mineral as the fixed effect, generation as the time factor and dam as the subject. The variable mineral requested different intercepts for different minerals, generation modeled an overall linear growth trend, and mineral X generation interaction made the slopes different over time. Heterogeneous slopes were tested by including generation in the model. Because there were 21 comparisons of mineral types, multiple comparison procedures were used. Hsu (1996) stated that multiple comparison procedures can be categorized by the strength of inference they provide. He wrote that these tests can provide the types of individual, inhomogeneity, inequalities and interval inferences. Among those, interval inference gives the strongest inferences. Tukey's test (Tukey, 1953) gives interval type inferences and is more powerful than Bonferroni, Sidak, or Scheffe methods for pairwise comparisons (SAS, 1999). Difference tests were adjusted by Tukey (1953) method. Since the data was unbalanced, adjustments were approximated using a method defined by Kramer (1956).

Ratio of males in a province was calculated by dividing the number of males born in that province to the total number of people born. Proc mixed procedure of SAS V8 (SAS Institute Inc., 1999) was used for analyses. The model included province, year and province*year interaction as fixed effects and ratio of males born as the dependent variable. Because there were 66 comparisons of provinces, multiple comparison procedures were used. Difference tests were adjusted by Tukey (1953) method and adjustments were approximated using a method defined by Kramer (1956).

Human and Diets: Adults was calculated from dietary surveys by nutritional habits in Çanakkale. The surveys were conducted in healthy and free-living populations, in both sexes (m/f). Data were obtained from Çanakkale Statistical Health Unit, Ministry of Health.

Table 1: Estimated Minerals Requirements of adult Mice and Human

Mouse** (g/kg/) Minerals	Amount, per kg diet	Human (mg -µg /day)*
Calcium	5,0	1000
Chloride	0,5	750
Magnesium	0,5	2-5
Phosphorus	3,0	700
Sodium	0,5	500
Potassium	2,0	2000
Iron	35,0	8
Manganese	10,0	2-5
Zinc	10,0	10-12
Iodine	150,0	150-150
Molybdenum	150,0	75-250 (µg)

** Adapted from Nutrient Requirements of Nonhuman Primates.

* Adapted from Linus Micronutrient Information Center, Oregon State Univ.

Results

An overall curve accounting for possible heterogeneous slopes was tested by including generation in the model and it was significant (Table 2). Mineral type was included to test the null hypothesis of a common intercept, and this hypothesis was significant also. Generation by mineral type interaction test revealed a small difference between the slopes ($P > 0.10$) of the mineral types. Dams given K had the highest male ratio in their litter followed by those given Ca+K (Table 3). Dams given Ca produced litters with the lowest male ratio. When no adjustments were made for multiple comparisons, differences between Ca and Ca+K,

Table 2: Tests of fixed effects in mice

Effect	F Value	P-Value
Mineral	2.75	0.035
Generasyon	3.97	0.049
Generasyon*Mineral	1.54	0.175

Table 3: Least squares means for different minerals in mice

Mineral	Estimate	Standard Error
K[a]	0.515	0.055
Ca+K[a]	0.514	0.055
Na[ab]	0.496	0.055
Control[ab]	0.460	0.055
Mix[ab]	0.411	0.055
NaK[ab]	0.401	0.055
Ca[b]	0.261	0.055

[a,b]Column values with different superscripts differ (P<0.05)

Table 4: Least Squares Means for different provinces

Province	Estimate	Standard Error
Eceabat	0.536	0.040
Merkez	0.524	0.013
Gokçeada	0.524	0.040
Biga	0.522	0.012
Gelibolu	0.518	0.017
Yenice	0.517	0.018
Ayvacik	0.516	0.020
Lapseki	0.515	0.018
Ezine	0.502	0.020
Çan	0.495	0.016
Bayramiç	0.469	0.018
Bozcaada	0.446	0.040

between Ca and K, between Ca and Na and between Ca and control were significant. However, when the multiple comparisons were adjusted using Tukey-Kramer method, only significant differences were between Ca and Ca+K and between Ca and K, which had the lowest and highest values of male ratios in a litter.

Discussion

Several studies published in past (Bolet *et al.,* 1982; Hewit, 1998) do not seem to be sufficient enough to explain the relationships between sex constitution and minerals consumed. In this study, minerals and sex ratios obtained from animals consuming different types of minerals were compared. Stolkowski and Choukroun (1982) has reported that the intervention of ions in ovarian metabolism is obtained by controlling the diet of the mice, the decisive factor being the ratio of K, in the daily diet. Data presented in Table 2 show that K group had the highest male while Ca group had the highest female ratio in their litter. This findings agree with previous reports for preconception selection of sex in

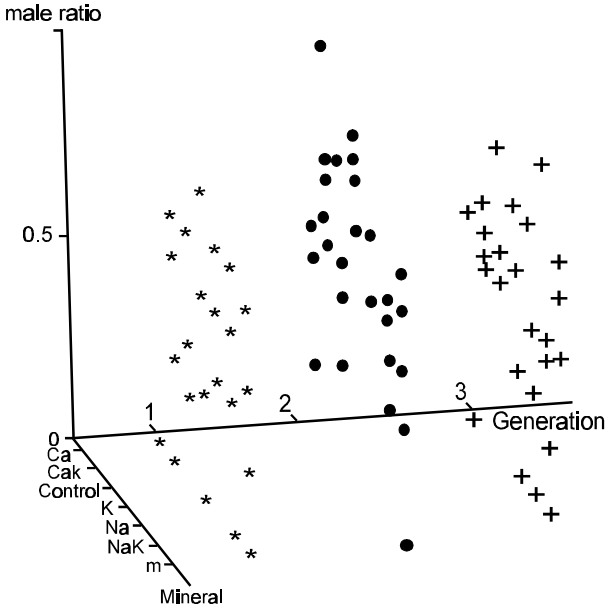

Fig. 1: Changes in male ratios by generation and mineral type in mice

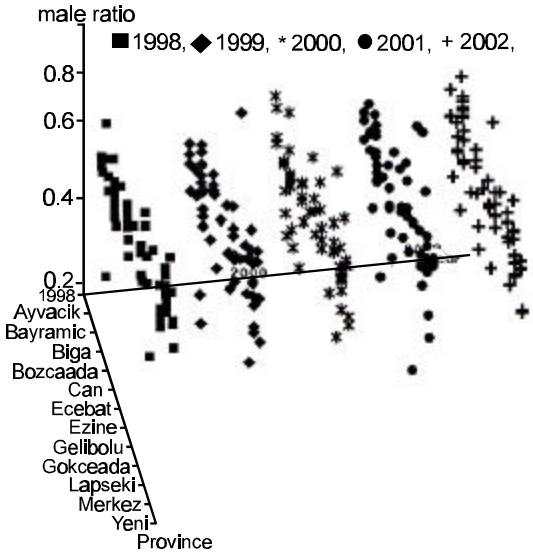

Fig. 2: Changes in male ratios by year and province in humans in Çanakkale

sows and man reported Bolet *et al*. (1982). Bird and Contreras (1986) has been reported that as dietary Na increased, the proportion of males decreased in rat litters. In this study presented here, when no adjustments were made, differences between Ca and Ca+K, between Ca and Na and between Ca and control were significant. However, when multiple comparisons were adjusted using Tukey-Kramer method, the only significant difference was between Ca and Ca+K, which had the lowest and highest values of male ratio in a

litter. In This study mice fed on a diet containing 6.39% Ca had significant values compared to other groups (P>0.10) as reported by Stolkowski and Lorrain (1982). Technology has not yet provided a routine method for separating the X- and Y-chromosome-bearing sperm (Hossain *et al.,* 1998) But previous researchers have suggested that dietary mineral content may affect the phenotypic sex ratio through changes in the genotypic sex ratio, or alternatively, via changes in the environment in which the genes are expressed. These findings are important for basic research concerning maternal nutrition and development (Bolet *et al.,* 1982; Hewit, 1998). Province with the highest male ratio was Eceabat and that with the lowest male ratio was Bozcaada (Table 4). Number of female births was higher for inland areas compared to areas closer to sea (Table 4). Bolet *et al.* (1982) suggested that in areas where dairy products are consumed, high Ca consumption results in higher number of female births. People inland tend to consume more dairy products compared to those living close to the sea and this maybe the reason inland areas have higher number of female births. Particularly fresh fruits, vegetables, fish, and unprocessed food consumers are in high numbers in this province and food rich in potassium is widespread in Eceabat. On the contrary, consumption of processed food, cheese and fish is high in Bozcaada and food products rich in calcium is common in this province. Effects of year and province on the ratio of males were small (P>0.10). Differences between Bayramiç and Biga, Bayramiç and Gelibolu, Bayramiç and merkez were significant when no adjustments were made for multiple comparisons. When the differences were adjusted for multiple comparisons using Tukey's method, none of the differences were large enough to be significant. All differences among years were small (P>0.10) regardless of the adjustment.

Results of the present study indicated that parents fed Ca rich rations tended to have female progeny. However, provinces of Canakkale did not differ significantly for the number of males born; different provinces had similar gender ratios regardless of the location and feeding rations. The results warrant further research in this area.

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