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Comparing the Effects of Sodium and Potassium Diet with Calcium and Magnesium Diet on Sex Ratio of Rats' Offspring

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Abstract: Sex determination has scientific basis for prevention of genetic sex linked diseases in addition to social backgrounds. There are many methods for sex determination. This study was designed to investigate the effects of adding different ions to the drinking water of rats on determination of rats' offspring sexes. In total, 72 female Vistar rats were chosen and randomly divided into three groups. Group one (NaK) was supplied with sodium and potassium diet, group two (CaMg) was supplied with calcium and magnesium diet, and group (C) as the control group. After 15 days of special diet, the rats were mated with male rats and were separated after pregnancy. The newborn rats' sexes were determined after delivery. The data were entered and analyses by SPSS software using t. test. In CaMg group, 66 male and 97 female rats were born (sex ratio = 0.68), while these rates were 91 male and 73 female (sex ratio = 1.25) in the NaK group. In the control group, 72 male and 75 female rats were born (sex ratio = 0.96). The difference in the sex ratio between NaK and CaMg group was statistically significant (p-value = 0.007). While the differences between NaK group with control group (p-value = 0.251) and between CaMg group with control group (p-value = 0.133) were not statistically significant. Our results suggest that different amount of ions in the diet of rats can have significant effect on the sex ratio of delivered rats' offspring.

Key words: Sodium, potassium, calcium, magnesium, sex ratio, vistar rat

Introduction

Sex determination has been the subject of many human studies for a long time. Advances in genetic showed that some genetic diseases are sex linked and only occurs in certain sexes. Therefore, sex determination found scientific basis in addition to social backgrounds.

The sex is determined by the genetic elements of the sperm, but it is unclear that which factors decide whether a sperm carrying a Y or an X chromosome will fertilize the egg. It is believed that sperm carrying a Y chromosome had higher motility while sperm carrying an X chromosome are believed to be more resistant.

Overall, in human population, the ratio of male to female at birth is nearly equal to one (1.06). Countries with reliable demographic data report sex ratio at birth (SRB) of around 105 to 106 male births for every 100 female births.

Different race/ethnic groups have different SRB. For example, Blacks have lower SRB than Whites, while Whites have lower SRB than Asians in the U.S., especially Chinese and Filipinos (Jacobsen *et al.,* 1999). These differences are likely due mainly to the genetics background of different ethnic groups.

Human influence can change the SRB. For example, the SRB for the U.S. are biologically normal and are not influenced by human interventions. In contrast, the SRB for Taiwan, China and South Korea are higher than biologically normal, and likely represent various kinds of human interventions (Jacobsen *et al.,* 1999).

There are different methods for sex selection. Overall these methods can be divided into three different kinds;

1. Preconceptual methods such as flow cytometric separation of X and Y sperm are about 85% effective at producing a girl, and 65% effective in producing a boy (Jacobsen *et al.,* 1999; Jimenez *et al.*, 2003).
2. Periconceptual methods are based on the observation that conception close to ovulation is more likely to result in a boy. Other methods include positioning during intercourse, vaginal douching, and so on. The effectiveness of these methods has not been well documented.
3. Postconceptual medically assisted sex selection is possible by employing prenatal testing (chorionic villus sampling, amniocentesis, ultrasound) and termination of pregnancy. In-vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD), provide an alternative which does not require abortion. After IVF, embryo biopsy is performed on Day 3 at the 8-cell stage to remove one or two cells for genetic examination. PGD can be used to detect chromosomal abnormalities (by fluorescence in situ hybridization [FISH]) and single gene disorders such as thalassemia or haemophilia (by DNA probe or polymerase chain reaction [PCR] amplification of specific DNA sequences).

There are many studies trying to investigate the efficacy of theses methods. Experiments on mouse showed that

both pre- and post-conceptional mechanisms of sex ratio distortion may explain, under different conditions, sex ratio deviations at birth (Jimenez *et al.*, 2003; Krackow and Burgoyne, 1997).

Some studies were based on theories that sperm carrying the X or Y chromosome favoured 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 and thereby affect the sex of the offspring. While, in one study, rabbit semen was collected, diluted 1:10 with a buffer of pH 5.4, 6.9, or 9.6, and after 20 minutes 0.5 ml of semen-buffer mixture was used for insemination in an ovulation-induced female. Newborn pups were examined both externally and internally for gender. The females inseminated with acidic semen had 6 litters, 50 offspring, with 48% males; those with neutral semen had 8 litters, 48 offspring, with 63% males; and those with alkaline semen had 7 litters, 49 offspring, with 49% males. There was no significant difference in these sex ratios from the expected 50% males (Muehleis and Long, 1976).

Krackow examined the effects of timing of mating and crowding of pregnant females on litter sex ratios in mice. Females either copulated during periods when no other female of the mating unit copulated simultaneously (single mating condition) or when more than one female copulated (multiple matings condition). Two crowding conditions were imposed on the animals: the females were placed into individual cages after mating (isolated condition), or they remained in the group until shortly before littering (crowded condition). The results showed a reduced sex ratio variance under single mating and crowded conditions (Krackow, 1997).

There are also methods which use different food combinations and especial diets to maximize the chance of having a baby with specific sex. The old believe is that eating salty, savoury foods leads to delivering a boy and calcium rich foods to a girl. Some believes that the ratio of the minerals sodium, potassium, calcium and magnesium are important in determination of baby's sex. It was shown that pregnant female house mice maintained on a consistent low-food diet give birth to a lower proportion of males than do control females fed ad libitum (Meikle and Thornton, 1995).

A study was undertaken to assess whether the use of clomiphene citrate in conjunction with albumin-separated sperm would alter the sex ratio towards females and, if so, whether this skewing was due solely to the induction of ovulation. The results showed that clomiphene citrate in conjunction with albumin-separated sperm decreased the sex ratio but this reduction was not exclusively due to induction of ovulation (Silverman *et al.*, 2002).

It is quite likely that, in the feature, parents will be able to use genetic engineering to select the sex of their child by directly manipulating the sex of an embryo. Some might think that this method would be a more ethical method of sex selection than present technologies such as PGD because, unlike PGD, it does not need to create and destroy embryos (Matthew Liao, 2005).

In this experimental prospective study, the effects of adding monovalan ions (sodium and potassium) and divalan ions (calcium and magnesium) to the drinking water of rats on determination of rats' offspring sexes was investigated.

Materials and Methods

In total, 72 female 10 weeks old Vistar rats (purchased from Razi Institute, Tehran, Iran), were chosen and randomly divided into three groups of 24 rats each. Four rats from each group were separated and the following protocol was applied on them. The protocol was repeated six times, with the intervals of two months, to include all the rats in each group. Group one (NaK) was supplied with drinking water mixed with 1% sodium and potassium, group two (CaMg) was supplied with drinking water mixed with 1% calcium and magnesium diet, and group C was chosen as a control group and pure drinking water was given to them. After 15 days, the rats were mated with male rats and were separated after they were pregnant.

Females inseminated in each session were housed together in 26.5 * 20.5 * 13.5-cm plastic cages until 17 days post pregnancy. At this point, pregnant females were housed individually and examined twice a day until parturition. Within the first 12 h after parturition, the number of litters and the gender of pups were recorded. Pups were sexed by means of the ano-genital distance, which is longer in males (Tarin *et al.*, 1999); this was confirmed in later examinations during preweaning development. The data were entered and analyses by SPSS software using t.test and ANOVA. The p-value less than 0.05 was considered as significant.

Results

In group NaK, under sodium and potassium diet, 23 rats out of 24 became pregnant which delivered 164 offspring. Their gender was 91 male and 73 female (55.5% male and 44.5% female). In group CaMg, under calcium and magnesium diet, all of the 24 rats became pregnant and delivered 163 offspring. From all pregnant rats, 66 male and 97 female rats were born (40.5% male and 59.5% female). Finally, in the control group (group C) all of the 24 rats became pregnant and delivered 147 offspring. From all pregnant rats, 72 male and 75 female rats were born (49% male and 51% female).

Overall, the sex ratio in the calcium and magnesium group was 0.68, while this ratio was 1.25 in the group under sodium and potassium diet. In the control group, the sex ratio was 0.96.

The difference between the sex of offsprings between

Table 1: The sex ratio in different groups of studied rats

Group	Total no of offspring	Male offspring		Female offspring		Sex ratio
		no	%	no	%	
NaK	164	91	55.5	73	44.5	1.25
CaMg	163	66	40.5	97	59.5	0.68
Control	147	72	49	75	51	0.96

group NaK and CaMg was statistically significant (p-value = 0.007). While the differences between group NaK with control group (p-value = 0.25) and between group CaMg with control group (p-value = 0.133) were not statistically significant (Table 1).

Discussion

There are many methods for sex determination, that among them specific diet could be the method of choice because of its simplicity, low expenses and the public approval. The American Society for Reproductive Medicine has ruled that it is proper and ethical to help couples to choose the sex of their babies (Gottlieb, 2001).

In societies with heavy preferences for male children, when couples are no longer able to have the number and gender mix of children they desire, they will use various kinds of human interventions to maximize the chances of realizing their fertility desires.

In most societies around 105 boys are born for every 100 girls. This biologically normal level of 105 is likely an evolutionary adaptation to the fact that males have lower survival probabilities than females. Therefore, 105 or so males are required at birth for every 100 females for there to be around equal numbers of males and females when the groups reach the marriageable ages in their twenties.

An abnormally high SRB have been reported in Taiwan, China, South Korea and a few other countries such as India since the 1980s (Park Chai Bin and Cho Nam-Hoon, 1995; Nair, 1996; Poston *et al*., 1997; Arnold, 1997; Clark, 2000). In China since the mid-1980s, the SRB has been on the increase, reaching 114.2 in 1992 (Gu and Roy, 1995) and 116.3 in 1994 (Tianlu, 1997). According to figures published in 2002 in state run Chinese media, 116.86 boys are born for every 100 girls in China (Plafker, 2002).

South Korea shows the highest SRB of 117.2 in 1990. These higher SRBs are likely due to prenatal sex identification followed by sex-selective abortion, and the concealment of female births (Zeng *et al*., 1993).

The disadvantages of sex selection techniques had big media coverage. Therefore, infertility specialists have expressed concern that media coverage of this technique might have raised unrealistic suspicions about its potential to prevent sex linked inherited conditions (Mayor, 2001).

Medically assisted sex selection for non-medical reasons is banned in many countries such as the United Kingdom and Canada. Paradoxically, it is legal to attempt preconceptual sex selection by "natural" means, even if these employ technology developed specifically for this purpose (Jansen, 1998).

Overall many important ethical issues must be addressed about sex selection. For example, sex selection may be beneficial to the child born if parents will treat a child of that sex more favourably. Sex selection might also cause psychological harm if the procedure does not produce a child of the desired sex. The other issues include; the importance of parent-child relationships, couple relationships, the right of individuals or couples to choice and personal desires (Hollingsworth, 2005). Concerns about these issues should be addressed by scientific investigation. In addition, the mutagenic risks of the sex selection procedure to children born must be evaluated (Benagiano and Bianchi, 1999).

Public opinion about the sex selection is another important issue which was the subject of many researches so far. In order to assess opinions and concerns about preconception sex selection for non-medical reasons, a social survey has been conducted in Germany on 1005 men and women 18 years and older. The results showed that only a minority of 11% approved of the use of preconception sex selection for non-medical reasons, while most of them were against it. In total, 87% of respondents believed that 'children are a gift and deserve to be loved regardless of any characteristics such as beauty, intelligence or sex', 79% argued that choosing the sex of children is 'playing God' and 76% were opposed because it is seen as 'unnatural' (Dahl *et al*., 2004). Similarly, an opinion poll of more than 2000 people in the United Kingdom showed that more than 80% of respondents were against the use of sex selection techniques for non-medical reasons (Kmietowicz, 2003).

Various methods now exist for attempting to choose to have a baby of a desired sex.

With the recent advent of flow cytometric separation of X and Y sperm and PGD, couples no longer have to employ abortion to select sex. PGD offers the only sure way of determining the sex of the offspring. This method is expensive and also requires the use of IVF. Currently, the most common reasons to request sex selection is family balancing and in case of certain genetic diseases (X-linked diseases) and the diseases that are not sex linked but are more common in boys such as autism. (Ralph, 1997).

Most of the non-invasive methods involve making the conception environment more attractive for specific type of sperm. Among different methods for sex selection, specific diet is the method of choice because of its simplicity, low expenses and the public approval. Our results suggest that different amount of ions in the diet of rats can have significant effect on the sex ratio of delivered rats' babies. It seems that relative excess of sodium and potassium ions in the diet would favour the birth of males, while relative excess of calcium and magnesium ions in the diet would favour the birth of females. In a similar experiment on sows to check that if mineral imbalance in the diet of the female before fertilization affects the sex ratio of the progeny, out of a total of 677 births, the sex ratio was 55.7 with the sodium and potassium diet and 48.3 with the calcium and magnesium diet (Bolet *et al*., 1982). These results show that sodium and potassium diet is in favour of male birth, which is similar to our results. More studies on more cases and different species are needed to confirm these results. In one study on human, the intervention of ions in ovarian metabolism was obtained by controlling the diet of the woman; the decisive factor was the ratio of sodium and potassium to calcium and magnesium in the daily diet. High values of this ratio lead to boys, and low values to girls. Using this method since 1970 in 47 births, only 7 of them failed to produce the expected sex (Stolkowski and Choukroun, 1981). These results were confirmed by another study on 281 couples, who adhered to a diet specific in its amounts of calcium, magnesium, sodium and potassium. This dietary method of preconceptional selection of fetal sex resulted in the anticipated outcome in about 80% of the cases (Stolkowski and Lorrain, 1980).

In conclusion, our study showed the importance of special diet with the right proportion of different ions on determination of sex ratio in rats. Because of the safety of this method before and during pregnancy, it can apply to pregnant women to investigate the effects of different ions on the sex ratio of human beings.

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