Research in Obstetrics and Gynecology 2016, 4(1): 11-15
DOI: 10.5923/j.rog.20160401.03

Preconceptional Minerals Administration Skewed Sex Ratio in Rat Offspring

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Abstract ***Rational*:** Natural sex selection methods have been applied for several decades. Sex determination has scientific basis for prevention of genetic sex linked diseases like Duchenne muscular dystrophy and also, for personal, social, or cultural backgrounds. There are many methods for sex determination. ***Objective*:** Sex selection using (ART) Assisted Reproductive Technologies without doubt accurate and precise but unfortunately very expensive. So, this study was designed to assess the efficacy of adding different ions to the drinking water of rats on determination of rats' offspring sexes. ***Subject and Methods*:** This study was carried out using 60 sexually mature male and female rats Sprague-Dawley rats, 2 months old, from central farm for experimental animals of Vacsera, Giza, Egypt. They were housed in 12hrs dark and 12-hrslight and fed a standard rodent pellet diet to acclimate for two weeks. Rats were divided into three groups: Group one: (Control) was chosen as a control group and pure drinking water was given to them. Group two (Na & K) was supplied with drinking water mixed with 1% sodium and potassium, and Group Three: (Ca & Mg) was supplied with drinking water mixed with 1% calcium and magnesium. The proestrous female cohabited (1:1) nightly with male. On the next morning the presence of a vaginal semen plug enabled the designation of day zero of pregnancy. The inseminated females were caged together in plastic cages until 17 days post pregnancy. At this point, pregnant females were housed individually and examined twice a day until parturition. Within 12hrs after parturition, the number of litters and the gender of pups were recorded. Pups were sexed by means of the anogenital distance, which is longer in males. Also, pups were examined for external malformations and diameter of crown rump length. The data were entered and analysed by SPSS software using t. test. ***Results*:** The results showed that effect of these minerals resulted in (56.81% male and 43.19% female) in Na& K fed group. While, in (Ca & Mg) fed group, (39.20% male and 60.79 % female). Also, by examining the delivered pups no signs of congenital or morphological anomalies were observed. Both crown rump and body weight values revealed that there is no statistically significant difference between the control and both (Na & K) and (Ca& Mg) groups. Also, there was statistically significant difference in male anogenital distance (mm) between (Na & K) (Ca& Mg) group which skewed gender toward females. ***Conclusion*:** This study concluded that; rats fed on (Na & K) ratios tended to have male progeny and those whom fed (Ca& Mg) ratios tended to have female progeny. This study reflect the potential role of such minerals on gender preselection; it could be have significant effect on the sex ratio of delivered rats' offspring.

Keywords Gender selection, Na, K, Ca, Mg, Sex ratio, Male, Female

1. Introduction

Gender preselection desire of the human species to control the gender of its progeny prior to conception has always existed. With sex selection, as with many issues of emotional appeal, political positions have predated careful reflection, and legislative initiatives have marched well in advance of strategic planning. Several factors enhance parents for baby gender selection range from family balancing to culturally imposed preference for boys to prevention of sex-linked hereditary diseases [1, 2]. The idea that maternal diet could influence the sex of the offspring has received considerable interest of many scientists and clinicians worldwide long time ago.

Some of these clinical retrospective diet surveys carried out by Lorrain and Duc, who confirmed that for couples with three or more girls and no boys, calcium and magnesium was decidedly dominant in the mother's diet, whereas the mothers of offspring predominantly male exhibited a sodium intake above average [3, 4]. A possible connection between the diet of patients and the sex of their descendants has been postulated several times [5].

In UK a group of scientists surveyed maternal dietary in (740) women and has confirmed a predominance of sodium and potassium in the pre-conceptional mineral intake of

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Published online at http://journal.sapub.org/rog

mothers who bore boys [6].

Several investigators considered the hypothesis that, the mineral intake could skew the sex ratio. Thus, they have studied women who adhered to a pre-conception diet specific in its amounts of calcium, magnesium, sodium and potassium [3]. Between 75% and 80% of the mothers (n = 553) were successful in delivering a baby of the desired sex [7-11].

The balance between sodium and potassium versus calcium and magnesium could change the receptors of the oocyte wall to favor the attraction of either a male or female sperm. When there is a high sodium and potassium intake, and a low calcium and magnesium in the female's diet, the oocyte wall will change to attract the (y-sperm). While, more calcium and magnesium in the blood and a low sodium and potassium will attract the (x-sperm). The connection between mineral intake and sex ratio of the offspring was first observed in the rat during long-term experiments. The result was 68% in calcium and magnesium group and 70% in sodium and potassium group [12].

Another group of investigators reported the influence offspring's sex is by controlling the time between intercourse and ovulation. Y-bearing spermatozoa are supposedly slightly lighter and faster but also more fragile and shorter-lived than X-bearing spermatozoa [13].

Intercourse close to ovulation would therefore favor boys. This theory was originally put forward by Kleegmanand later popularized by Shettles [14, 15]. As the time of ovulation grows nearer, it is well known that the quality and quantity of the cervical mucus changes. Studies have shown that the viability, longevity, and motility of the "X" and "Y" chromosome carrying sperm are affected differently by these mucus changes. The utilization of these variables to create a preconception sex selection bias is dependent upon the ability to accurately predict when ovulation is to occur [2]**.** The selection for a male offspring requires that intercourse be performed as close to ovulation as possible. While, intercourse that occurs approximately three days prior to ovulation has a greater chance of conceiving a girl [16, 17].

Y sperm can, under ideal conditions, move more quickly than the X sperm. The X sperm are hardier than the Y sperm and are more resistant to various forms of stress [13]. Position of intercourse and depth of penetration are important. The man should try for deep penetration at the time of his climax will help deposit the sperm closest to the cervix, where secretions are more favorable to the male-producing sperm. Thus, for couples interested in male it recommended that vaginal penetration from the rear (dog style position) and shallow penetration by the male at the time of his orgasm will further help ensure that the sperm passes through the vaginal canal, where secretions are naturally more acidic and therefore, in a relative sense, favor the female-producing sperm [15, 17].

Vaginal environment and cervical mucus can greatly influence the chances that either an "X" carrying sperm or "Y" carrying sperm will be successful in fertilizing the egg. Vaginal douche should be utilized approximately 30 minutes prior to intercourse on the day of ovulation in male preconception kit. While, in female preconception kit, it is important to douche approximately 30 minutes prior to the last episode of intercourse occurring approximately three days prior to the time of ovulation [2]. The main objective of the present study is to assess the efficacy of a maternal diet in combination with timing of intercourse, vaginal douche and sex position before ovulation as a method to improve the chances of conceiving a girl or boy.

2. Materials and Methods

Animals and Mating:

This study was carried out using 60 sexually mature male and female rats Sprague-Dawley rats, 2 months old, from central farm for experimental animals of Vacsera, Giza, Egypt. They were housed in 12hrs dark and 12hrs light and fed a standard rodent pellet diet to acclimate for two weeks. Rats were divided into three groups: Group one: (Control) was chosen as a control group and pure drinking water was given to them. Group two (Na & K) was supplied with drinking water mixed with 1% sodium and potassium, and Group Three: (Ca & Mg) was supplied with drinking water mixed with 1% calcium and magnesium. The proestrous female cohabited (1:1) nightly with male. On the next morning the presence of a vaginal semen plug enabled the designation of day zero of pregnancy [18, 19]. The inseminated females were caged together in plastic cages until 17 days post pregnancy. At this point, pregnant females were housed individually and examined twice a day until parturition.

Maternal and Embryo-Fetal Study:

This maternal and embryo-fetal study was conducted in accordance with the U.S. Environmental Protection Agency TSCA (Toxic Substances Control Actz) Test Guidelines [18]. 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 anogenital distance, which is longer in males [20]; this was confirmed in later examinations during preweaning development. Also, pups were examined for external malformations and diameter of crown rump length. Heart blood was drawn and sera were separated and analysed using Willis methods for the determination of metals in blood serum by atomic absorption spectroscopy [21].

Statistical Analysis:

The statistical analysis of the obtained data was done and the analysis was revised by SPSS 12 for windows (2003).The Student's "t"-distribution were adopted for assessment of significant changes occurring between the groups.

3. Results

Prior to pregnancy rats were divided into three experimental groups the control administrated drinking

water, (Na & K) group were administrated (1% Na & K/100ml H_2O) and (Ca & Mg) group were administrated (1% Ca & Mg /100ml H_2O) in drinking water. Blood samples was analyzed for Na+, K+, Ca2+ and Mg2+ concentrations. The minerals administration was continued for 1 week after pregnancy after this time a second serum analysis (Postconceptional). The obtained data revealed that; the concentrations of blood minerals during preconceptional time showed increase when compared to control; this increase reached (+ 0.65 %, + 19.24 %, + 47.84 % and+ 112.22 %) for Na+, K+, Ca2+ and Mg2+ concentrations respectively. During postconceptional time the concentrations of blood minerals showed decrease; the percentage of such decrease reached (- 2.82 %, - 16.14 %, - 26.82 % & - 48.69 %) as shown in table (1).

In (Na & K) group, 20 pregnant rats delivered 169 offspring. Their gender was 96 male and 73 female (56.80% male and 43.19% female). In (Ca & Mg) group, the 20 pregnant rats delivered 176 offspring. 69 male and 107 female rats were born (39.20% male and 60.79 % female). While, in the control group the 20 pregnant rats delivered 182 offspring, 92 male and 90 female rats were born (50.54% male and 49.45% female). Overall, the sex ratio in (Na & K) group was (1.31), while this ratio in (Ca & Mg) group was reached (0.64). The differences between the sexes of offsprings in the experimental groups were statistically significant as shown in table (2).

In Utero-Fetal Exposure Effect:

By evaluating the teratogenic potential of the administrated mineral to the pregnant rats, it was found that no signs of congenital or morphological anomalies were observed. Recording of both crown rump and body weight values revealed that there is no statistically significant difference between the control and both (Na & K) and (Ca& Mg) groups. Also, by measuring of male anogenital distance (mm) in (Na & K) group was found (3.16 + 0.05) vs (3.14 + 0.04) for control which was statistically non-significant difference. While anogenital distance (mm) was skewed the sex ratio in (Ca & Mg) group toward females and was reached (2.99 + 0.10) with percentage of change reached (-4.77 %) as shown in table (3).

Table 1. Serum Minerals concentration of rats Preconceptional and postconceptional

Groups	Na (mmol/l)	K (mmol/l)	Ca (mmol/l)	Mg (mmol/l)
Control	142.61+2.33	4.52 +0.41	2.32 +0.13	0.90 +0.11
Preconceptional	143.55 +2.4	5.39 +0.40	3.43 +0.10	1.91 +0.12
% Control vs Preconceptional	+ 0.65 %	+ 19.24 %	+ 47.84 %	+ 112.22 %
Postconceptional	139.49 +2.1	4.50 +0.33	2.51 +0.12	0.98 +0.19
% Pre vs Postconceptional	- 2.82 %	- 16.51 %	- 26.82 %	- 48.69 %

Values are mean (mmol/l) + SD. % = percentage of change.

Table 2. Sex ratio data for rat embryos exposed to minerals In Utero Pre-and Postconceptional

Groups	Control	Na & K	Ca & Mg
Dose	--	1% Na & K/100ml H_2O	1% Ca & Mg /100ml H_2O
Litter size	182	169	176
Male offspring	92	96	69
% of Males	50.54 %	56.80 %	39.20 %
Female offspring	90	73	107
% of Females	49.45 %	43.19 %	60.79 %
Sex ratio	1.02	1.31	0.64

Table 3. Foetal dimensions of rat embryos exposed to minerals in utero

Groups	Control	Na & K Group	Ca & Mg Group
Dose	--	1% Na & K/100ml H_2O	1% Ca & Mg /100ml H_2O
Crown-rump (cm)	3.75 + 0.04	3.67^{ns} + 0.09	3.62^{ns} +0.11
% of Change	--	- 2.13 %	- 3.46 %
Body Weight (gm)	3.92 + 0.25	3.86^{ns} + 0.10	3.73^{ns} + 0.30
% of Change	--	- 1.53 %	- 4.84 %
Male Anogenital Distance (mm)	3.14 + 0.04	3.16^{ns} + 0.05	2.99^{**} + 0.10
% of Change	--	+ 0.63 %	- 4.77 %

4. 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 [22]. This study investigated the efficacy of (Na, K, Ca and Mg) as sex pre-selection factors. The results showed that effect of such minerals resulted in (Na & K) group, (56.80% male and 43.19% female). While, in (Ca & Mg) group, (39.20% male and 60.79 % female); when compared with the control group. Also, by examining the delivered pups no signs of congenital or morphological anomalies were observed. Both crown rump and body weight values revealed that there is no statistically significant difference between the control and both (Na & K) and (Ca & Mg) groups. Also, there was statistically significant difference in male anogenital distance (mm) between (Na & K) (Ca & Mg) groups which skewed gender toward females. Our results were in agreement with the results of Stolkowski and Choukroun who reported the effect of Na, K, Ca, and Mg on human offspring the result was 80% males [9]. Several authors have studied women who adhered to a pre-conception diet specific in its amounts of calcium, magnesium, sodium and potassium. Between 75% and 80% of the mothers were successful in delivering a baby of the desired sex [7-11]. Also, (Na, K, Ca and Mg) were supplemented to the rats; the results showed that 68% females in calcium and magnesium group and 70% males in sodium and potassium group [12]. More recently our work was supported by Chandraju and his colleagues who studied the effect of Ca and Mg on rats fed with (Ca, Mg) food yielded maximum numbers of male offspring (77), while rats fed with normal food yielded lowest numbers of male offspring (50) [5]. 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 [23]. So, the results of this study supported by results of Chandraju and his co-workers who indicated that parents fed (Ca + Mg) rich ratios tended to have female progeny. On the other hand, parents fed (Na & K) rich ratios tended to have male progeny [5]. Altering diet to include and exclude certain food, 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. 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 depending on which diet is adhered to. This method under scrutiny claims of 80% accuracy based on one clinical trial of only 260 mothers, the results were published in the international journal of Gynecology and Obstetrics in 1980. The male diet is high in salt and potassium but low in sodium, calcium, magnesium and contains alkali-forming foods.

Sex selection is the practice of using medical techniques to choose the sex of one's offspring. This study concluded that; rats fed on (Na & K) ratios tended to have male progeny and those whom fed (Ca & Mg) ratios tended to have female progeny.

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