

Regular Article
**Effect of calcium and magnesium in identification of
baby in highsugar mammals**

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Effects of calcium and magnesium in diet to determine baby gender in high-sugar mammals have been investigated. Diabetes mellitus is a chronic, widely spread disease in living species. Sex determination has scientific basis for prevention of genetic diseases in addition to social backgrounds. 60 rats (obtained from veterinary college Bangalore), were divided in to three groups in the ratio male to female 1:5. The first group was made diabetic with Ca and Mg, the second group was non diabetic with Ca and Mg, group third was control unit (Non-diabetic without Ca and Mg). It was found that the delivered offspring male to female ratios were 4:1, 3.77:1 and 1.05:1 for the first, second and third groups respectively. Also, it was found that non diabetic rats fed with normal food yields maximum numbers of offspring (220), while non diabetic rat fed with Ca and Mg yields 205 and diabetic rats with Ca and Mg yield lowest numbers of offspring (130).

Keywords: calcium, diabetes, magnesium, sex ratio, vistar rat

Pregnancy is a state that allows a life form to develop with the support and protection of its mother's body. The growth and development of the fetus in gestation is partially determined by the genome of the fetus, which produces its own growth factors as well as the majority of its hormones. However, this genetic influence is highly dependent upon interaction with environmental factors (Van Assche et al., 2001). One environmental factor vital in the growth and development of the fetus is nutrition. The fetus is solely dependent on the mother to supply its nutrients. It is also dependent on the placenta, an essential organ in pregnancy, to transfer these nutrients from the maternal system to its own. Thus the fetal nutrition is a reflection of that of the mother's. This interaction exists in a sensitive equilibrium; if disturbed, there are fetal developmental consequences (Van Assche et al., 2001). Pre-

selection of the gender of offspring is a subject that has held man's attention since the beginning of recorded history. Although scientific studies on genes have been conducted recently, sex selection and gender preference have been considered since ancient time. Anaxagoras, a reek scientist was the first person who related the sex of fetus to testis (Mittwoch, 2005). People intending to conceive may want to select the sex of their children. The reasons for wanting a child of a particular sex include:

- 1) To avoid passing on a serious genetic condition which is associated with, or inherited through, a particular sex, for example, haemophilia, a sex-linked genetic condition which mostly affects males
- 2) To ensure that a child to be born is the opposite sex to existing children in a family, sometimes referred to as 'family balancing'

3) To fulfill personal or cultural preferences for a child of a particular sex. Gestational diabetes mellitus (GDM) is a frequent complication of pregnancy, affecting 3.5% of pregnancies in the United States (Engelgau et al., 1988). Because obesity and age is major risk factors for GDM, (Feig et al., 2002) the prevalence of GDM is increasing (Solomon et al., 1997). Although GDM may represent a previously unrecognized state of continuous hyperglycemia (ie, diabetes), most women with GDM show glucose intolerance that does not persist after pregnancy. Women with GDM have been shown to be more insulin-resistant than normal pregnant women, and their insulin secretion is defective relative to the degree of Insulin resistance (Xiang et al., 1999). There is a strong association between pregnancy in women with any form of diabetes and high infant mortality and morbidity in their offspring (Stenninger et al., 1998). Over the past 25 years, the rate of neonatal mortality among infants of diabetic mothers has declined from 250 per 1000 live births to approximately 20 per 1000 live births. About half of these deaths are due to congenital malformations that result from the diabetic intra-uterine environment (Weintrob et al., 1996). The frequency of congenital malformation, as well as morbidity associated with maternal diabetes, is directly related to the severity of the diabetes (Ornoy et al., 1998). Abnormalities in systems such as the cardiovascular system, musculoskeletal, and central nervous system occur 5 times more often in the offspring of diabetic mothers. Rare abnormalities like sacral agenesis and caudal regression syndrome occur between 200 and 400 times more frequently than the non-diabetic population (Weintrob et al., 1996). There are many Methods of sex selection such as: The consumption of particular foods, the use of various vaginal douches and the timing of intercourse in relation to ovulation, Sperm sorting, Pre-implantation genetic diagnosis (PGD), Selective abortion, Infanticide,

Periconceptual methods, postconceptual methods.

There are also methods which use different food combinations and especial diets to maximum 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 gender. 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 et al., 1995). In this study, We induce experimental diabetes with Streptozotocin to study the effects of adding di-valent ions (calcium and magnesium) to the drinking water of rats, offspring sexes was investigated.

Materials and Methods

Medicine

Streptozotocin or Streptozocin or Izostazin or Zanosar (STZ) is a synthetic antineoplastic agent that is classically an anti-tumor antibiotic and chemically is related to other nitrosureas used in cancer chemotherapy. Streptozotocin sterile powders are provided and prepared as a chemotherapy agent. Each vial of sterilized Streptozotocin powder contains 1 g. of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[(methylnitrosoamino) - carbonyl]amino]-D-glucopyranose and 200 mg citric acid. Streptozotocin was supplied by Pharmacia Company. Streptozotocin is available for intravenous use as a dry-frozen, pale yellow, sterilized product. Pure Streptozotocin has alkaline pH. When it is dissolved inside the vial in distilled water as instructed, the pH in the solution inside the vial will be 3.5-4.5 because of the presence of citric acid. This material is prepared in 1 g vials and kept in cold store and refrigerator temperature (2 - 8 °C) away from light. Control animals were given an equivalent volume of citrate buffer solution.

Animals

60 Adult female Wistar weighting 100-130g (70-80 days old) and still in their reproductive phase, were kept under constant and had free access to tap water. Before the start of the experiments, the rats were allowed to adapt for 1 week. The animals were then weighed, and a blood sample was taken from the tip of the tail for the determination of glucose and insulin levels. Vaginal wet smears were made to determine the estrous cycle of the rats. On the evening before estrus, female rats were housed overnight with male rats; the presence of spermatozoa in a vaginal smear the next morning was defined as day 1 of pregnancy.

Experimental design

To Induction of diabetes, Rats were fasted for 12-h before inducing diabetes, 20 adult Wistar rats weighting 100-130g (75-90 days old) were used for inducing diabetes. The rats were injected by a single intraperitoneal streptozotocin at the dose of 40 mg/kg of the body weight. STZ was freshly dissolved in 0.05 M citrate buffer, pH 4.5. For the i.p. injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using povidone-iodine solution and the designated amount of STZ was injected within 10 'after preparation in the caudal abdominal cavity using sterile 25g insulin needle. Streptozotocin induces diabetes within 3 days by destroying the beta cells (Karunanayake., 1975). Tail blood was collected for glucose determination using a glucometer (Accutrend Glucose, Roche Diagnostics, and Mannheim, Germany). Blood glucose levels were measured on the third day, STZ injected rats with blood glucose levels 15 mmol/l (270mg/dl) as well as polydipsia, polyuria and polyphagia for at least one week were considered to be diabetes (STZ rats). 20 Control animals [non diabetic (Ca, Mg) and 20 neither diabetic nor (Ca, Mg)] were injected with an equal volume of citrate buffer solution.

Diabetic rats and non-diabetic control group were kept in metabolic cages

individually and separately and within 15 days, on the specified diets (Ca, Mg and non Ca, Mg) feeding and metabolism control (15g Ca /kg and 1.5 g Mg /kg)[Table 1].the first group diabetics(Ca, Mg)and second group non diabetics(Ca, Mg)was supplied with drinking water mixed with 1% calcium and magnesium, the third group was chosen as a control group neither diabetics nor Ca, Mg, pure drinking water was given to them. After 15 days, on the specified diets, the rats at the estrus stage of the reproductive cycle were caged with male rats for mating and gestational day 1, was confirmed on the observation of a vaginal plug. At postnatal day 2, 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 pre weaning development. The data were entered and analyses by SPSS software using t test and the p-value less than 0.05 were considered as significant.

Results

It was found that, the first Group diabetic mothers (Ca, Mg), 16 rats out of 20 became pregnant which delivered (130) offspring. Their gender was 26 male (20%) and 104 female (80%). In the second group, non diabetic (Ca, Mg), all of the 20 rats became pregnant and delivered 205 offspring their gender were 43 male (21%) and 162 female (79%) and in the third group, neither diabetic nor (Ca, Mg) all of the 20 rats became pregnant and delivered 220 offspring that 113 male(51.36%) and 112 female(48.64%) (Table1).The sex ratio of male to female In the first Group of diabetic mothers (Ca, Mg) and In the second group, non diabetic (Ca, Mg) were 4:1-3.77:1, while this ratio in the third group, neither diabetic nor (Ca, Mg) was 1.05:1 respectively (Figures 1 and 2).The percentage of the female offspring of diabetic mothers (Ca, Mg) [80%] was higher than the female offspring in control group [48.64%] and also the percentage of female the offspring of non diabetic mothers (Ca, Mg) [79%] was

higher than the female offspring in control group [48.64%] (Figure 3). The difference in the sex ratio between the first Group of diabetic mothers (Ca, Mg) and the second group of non diabetic mothers (Ca, Mg) was not statistically significant (p-value - 0.03). While the differences between the Group of diabetic mothers (Ca, Mg) with control group (p-value - 31.45) and between groups non diabetic mothers (Ca, Mg) with control group (p-value -39.65) were statistically significant (Table 2). The Total

no of offspring in the first group of diabetic mothers (Ca, Mg) (130, 23.42%) was lower than Total no of offspring the second group, non diabetic (Ca, Mg) [205, 36.94%] and also in the third group, neither diabetic nor (Ca, Mg) [220, 39.64%]. (Figure 4) Body weight in STZ-induced diabetes group increased from 115±15g to 130±15g, while that of the control group increased from 115±15g to 265±13g on the day of experiment.

Table 1: Estimated Minerals Requirements of adult Mice and Human

Mouse** (g/Kg) Minerals	Amount Per Kg diet	Human*(mg-ug/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(ug)

**Adapted from Nutrient Requirements of Nonhuman Primates.

* Adapted from Lanus Micronutrient information Center, Oregon State Unit.

Table 2: Sex ratio in different groups of rats

Group	Total no of offspring	No. of male offspring	% age of male offspring	No. of female offspring	%age of female offspring	Sex ratio
Diabetic with Ca& Mg	130	26	20	104	80	4.00
Non-diabetic with Ca& Mg	205	43	21	162	79	3.77
Neither diabetic nor with Ca& Mg	220	113	51.36	112	48.64	1.05

Discussion

Pre-selection of the gender of offspring is a subject that has held man's attention since the beginning of recorded history. While the natural sex ratio at birth

is usually 104-107 male to 100 female (Visaria, 1901; Bose, 1951; Allied, 1967). in Chinese census data show that 20 years ago there were 108 male under the age of 5 for every 100 female, and that by 2000 this ratio

had shifted to 117 male to 100 female, with some regions reporting ratios of 130. In the Indian census of 2001 the sex ratio in the age group 0-6 was 117.8, with some northern states such as Punjab having ratios

as high as 120-125 (Bhaskar et al., 2007). These trends are mirrored in other Asian countries such as South Korea and Taiwan, which have sex ratios at birth of 108 and 109 respectively.

Figure 1: Sex ratio of Male and Female in different groups of rats

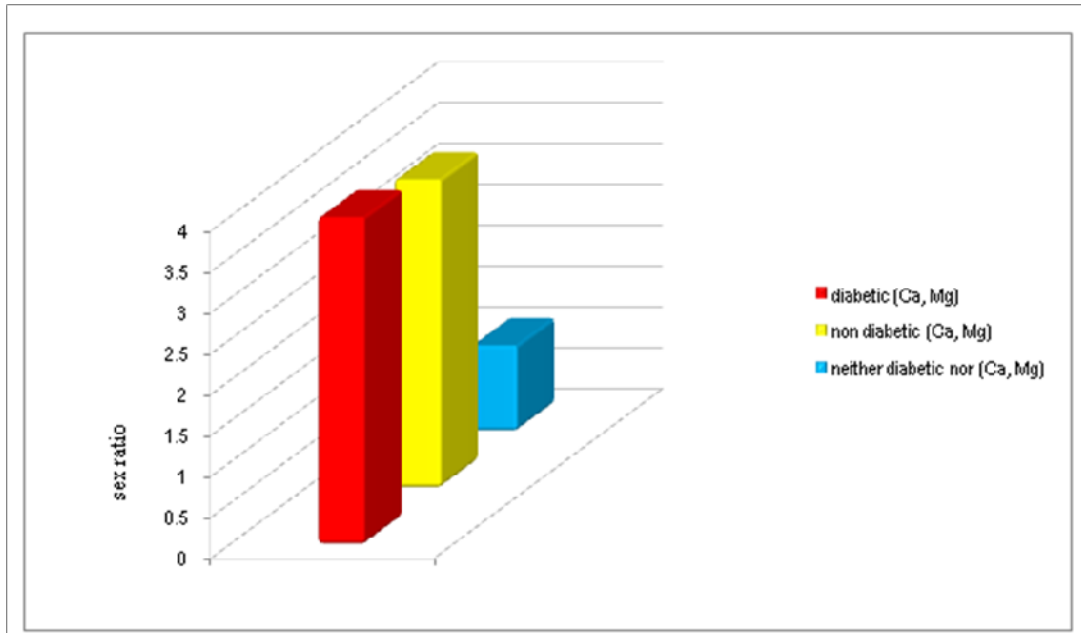


Figure 2: Comparison of Male and Female offspring in different groups of rats

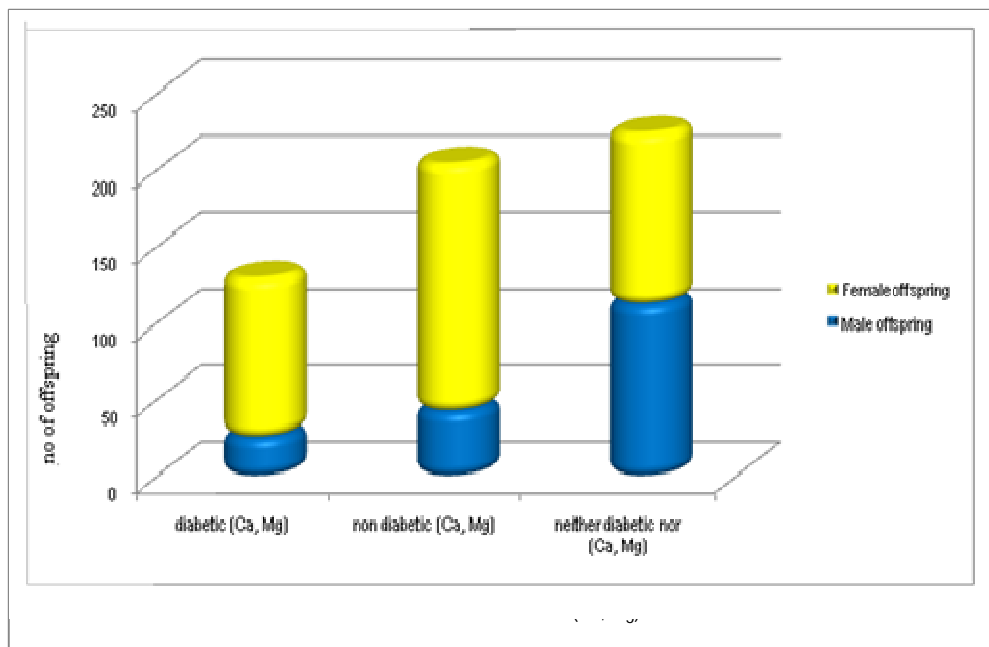


Figure 3: Percentage of Offspring sex in different groups of rats

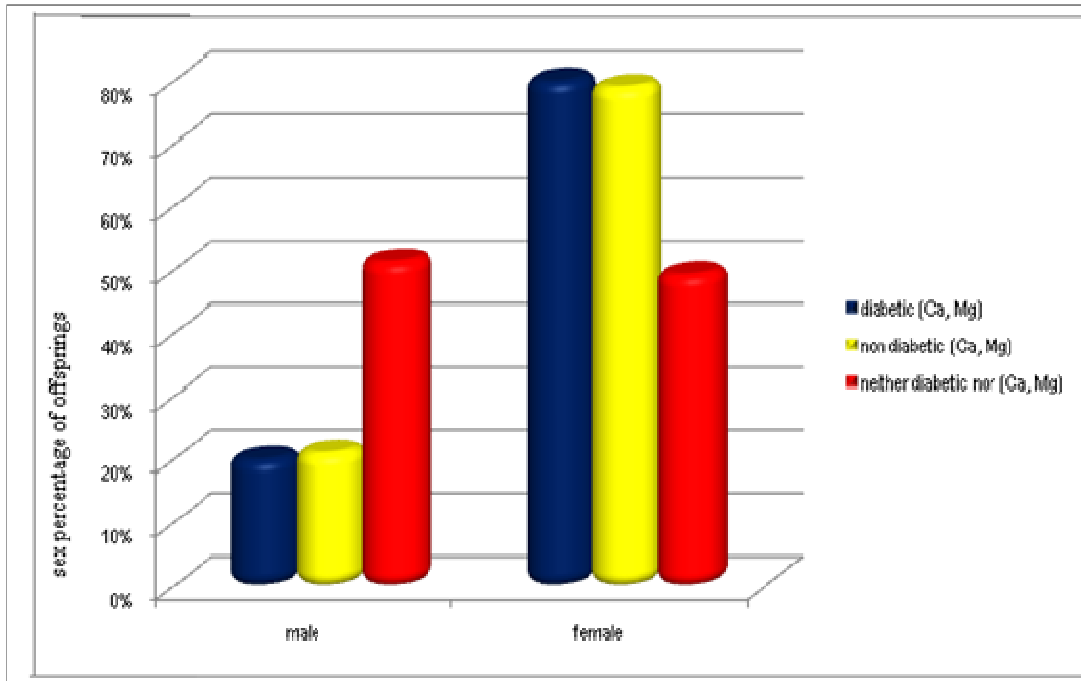
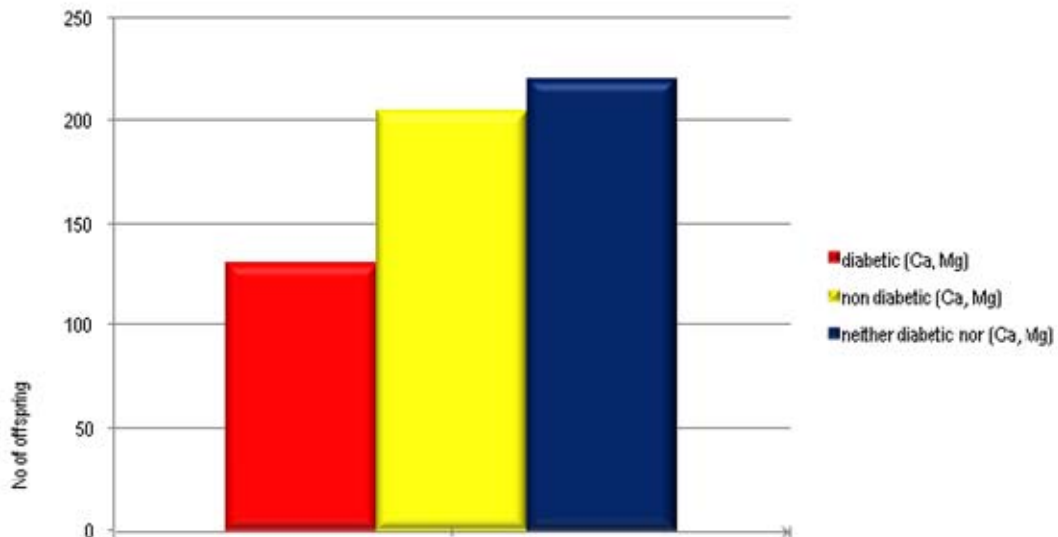


Figure 4: Number of offspring in different groups of rats



There are many reasons for sex determination that the strongest predictor of uneven sex ratio for a given parity is the sex composition of previous children (Retherford, 2003; Jha, 2006; Abrevaya, 2009). For families without a son, the higher the parity, the higher the probability of having a male as the next birth. The propensity to use sex selection increases with socio-economic status, especially education, and the proportion of male to female is larger in cities than in rural areas (Retherford, 2003; Jha et al., 2006). Despite a large demographic literature on the relation between male preference and fertility stopping behavior (Clark, 2000), there is little formal analysis of the link between fertility and sex selection (Park et al., 1995). For India, it has been argued that fertility decline increases the bias against female (Gupta et al., 1997), but the stated preference for male also appear to decline with lower desired fertility (Bhatand et al., 2006). In Korea simulations suggested that introduction of sex selection changed family size little, but did result in abortions of female fetuses equal to about five percent of actual female births (Park et al., 1995). For China allowing a three-child policy has been predicted to increase the fertility rate by 35 percent, but also reduce the number of female aborted by 56 percent (Ebenstein et al., 2009). Most of the sex selection in China is due to parents' with low levels of education (Ebenstein et al 2009). Data presented in table 4 show that non diabetic (Ca, Mg) group and diabetic (Ca, Mg) group had the highest female while neither diabetic nor (Ca, Mg) Group there was no different between their sex. This finding agrees with reports of preconception of sex in sows and man reported (Bolet et al., 1982). Results of this research indicated that parents fed (Ca + Mg) rich ratios tended to have female progeny. Sex selection is the practice of using medical techniques to choose the sex of one's offspring. Those are like the consumption of particular foods, the use of various vaginal douches and the timing of intercourse in relation to ovulation is some of the many methods

believed to influence whether a female or a male is conceived, and some techniques include sperm sorting, PGD, and selective abortion. Sex-selection procedures can be divided into two analytical categories:

(1) Procedures done for medical reasons; and (2) procedures done for non-medical, elective reasons. While there is some debate among doctors, ethicists, and the general public about the level of medical necessity that should justify a sex selection procedure, most accept that sex selection for medical reasons is beyond ethical reproach, and in some situations, should even be encouraged (<http://www.acog.org/>). However, elective, non-medical sex-selection, which is often performed for social or financial reasons, is the subject greater scrutiny and impassioned ethical debate.

Currently, doctors and geneticists are able to diagnose more than five hundred separate medical conditions in a developing fetus (Robert, 1995). Among these conditions are devastating genetic diseases such as hemophilia, Down syndrome, cystic fibrosis, Huntington's disease, (see Amy Harmon, 2007) and Hunter syndrome. For many parents who know they are genetic carriers of a particular sex-linked disease (such as hemophilia), sex selection can increase the likelihood that their child will be born healthy. This is the essence of medical sex selection. Non-medical sex selection procedures, on the other hand, are undergone for a variety of reasons, but few are as clear-cut as those cited for medical sex selection. As noted by the Ethics Committee of the American Society for Reproductive Medicine (ASRM); there are at least four prominent motivations that have historically prompted prospective parents to desire a child of a particular gender: 1) a desire to bear and raise children of the culturally preferred gender, 2) to achieve gender balance among children in a given family, 3) to determine a gendered birth order, and 4) to ensure the economic usefulness of offspring within the family (Judith, 2005). The consumption of particular foods, the use of various vaginal

douches and the timing of intercourse in relation to ovulation are some of the many methods believed to influence whether a female or a male is conceived. There is little evidence that these methods significantly alter the ratio of male to female births.

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 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. This is also consistent with the oriental philosophy that everything has a yin or yang quality and the foods supplied in the female diet, female and acid are all yin. The female diet is high in calcium but low in salt and potassium, containing acid 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 type of sperms to penetrate the depending on which diet is adhered to. Langendon and Proctor first published 'the preconception Gender Diet 'based on results reported (Stolkowski, 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 female diet is high in calcium but low in salt and potassium, containing acid forming foods. The diets nutritional content is questionable and contains multiple warnings.

It is recommended that you seek the advice of your 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 type of sperms to penetrate the egg depending on which diet is adhered to. The aim of this study was to elevate relationship between minerals and sex ratio in mice and human.

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