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Impact of Calcium and Magnesium Ions in Identification of Baby Gender in High-Sugar Hamsters

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Abstract
Impact of calcium and magnesium in diet to determine the baby gender in high-sugar hamsters has 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.30 hamsters (obtained from veterinary college Bangalore), were divided in to three groups at the rate 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 offsprings female to male ratio were 3.6:1, 2.61:1 and 1.04:1 for the first, second and third groups respectively. Also, it was found that non diabetic fed with normal food yields maximum numbers of offspring (90), while non diabetic fed with Ca and Mg yields 83 and diabetic with Ca and Mg yield lowest numbers of offspring (70).

Keywords: calcium, diabetes, magnesium, sex ratio, hamster, offsprings

INTRODUCTION

Pregnancy is a state that allows a life form to develop with the support and protection from 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 [1]. 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 [1]. Preselection 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 Greek scientist was the first person who related the sex of fetus to testis [2]. People intending to conceive may want to select the sex of their children. The reasons for wanting a child of a particular sex include:

a) To avoid passing on a serious genetic condition which is associated with, or inherited through, a particular sex, for example, hemophilia, a sex-linked genetic condition which mostly affects males
b) To ensure that a child to be born is the opposite sex to existing children in a family, sometimes referred to as 'family balancing'
c) 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 [3]. Because obesity and age are major risk factors for GDM [4] the prevalence of GDM is increasing [5]. Although GDM may represent a previously unrecognized state of continuous hyperglycemia (i.e., 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 [6]. There is a strong association between pregnancy in women with any form of diabetes and high infant mortality and morbidity in their offspring [7]. 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 [8]. The frequency of congenital malformation, as well as morbidity associated with maternal diabetes, is directly related to the severity of the diabetes [9]. 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 [8]. 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 maximize the chance of having a baby with specific sex. The old believe is that eating salty, savory foods leads to delivering a male and calcium rich foods to a female. 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 [10].

As a part of our ongoing research, we studied the Role of Sodium and Potassium ions in identification of baby gender in High-sugar mammals [11,12]. In the present study, we induce experimental diabetes with Streptozotocin to study the effects of adding mono-valent ions (calcium and magnesium) to the drinking water of hamster, offspring sexes was investigated.

FIELD EXPERIMENT AND SAMPLING

Streptozotocin or Streptozocin or Izostazin or Zanosar (STZ) is a synthetic antineoplastic agent that is classifically 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 1g vials and kept in refrigerator (2-8 °C) away from light. Control animals were given an equivalent volume of citrate buffer solution.

30 Adult female hamster weighting 150-180g (42-56 days old) and still in their reproductive phase , were kept under constant conditions of light (12 h light-dark cycle) and humidity, fed with standard laboratory chow ad libitum (Trouw, Gent, Belgium), and had free access to tap water. Before initiation, the hamsters were allowed to adapt for one week. Hamsters were then weighed, and blood sample was tested for glucose and insulin levels. Vaginal wet smears were made to determine the estrous cycle of the hamsters. On the evening before estrus, female hamsters were housed overnight with male hamster; the presence of spermatozoa in a vaginal smear the next morning was defined as day one of pregnancy.

To Induction of diabetes, hamsters were fasted for 12 h before inducing diabetes, 10 adult hamsters weighting 150-180 g (42-56 days old) were used for inducing diabetes. The hamsters were injected by a single intra-peritoneal 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 hamsters 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. Streptozotocine induces diabetes within 3 days by destroying the beta cells [13]. 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, STZinjected hamsters with blood glucose levels 15 m mol/l (270mg/dl) as well as polydipsia, polyuria and polyphagia for at least one week were considered to be diabetes (STZ rats).10 control hamsters [non diabetic (Ca, Mg) and 10 neither diabetic nor (Ca, Mg) were injected with an equal volume of citrate buffer solution.

Diabetic hamsters and non-diabetic control group were kept in metabolic cages individually and separately and within 16-21 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 supplied. After 16-21 days, on the specified diets, the hamsters at the oestrus stage of the reproductive cycle were caged with male hamsters for mating and gestational day 1, was confirmed on the observation of a vaginal plug. At postnatal day two, 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 [12]; 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 AND DISCUSSION

It was found that, in the first group diabetic mothers (Ca, Mg), all of the 10 hamsters became pregnant which delivered 70 offspring. Their gender was 15 male (21.43%) and 55 female (78.57%). In the second group, non diabetic (Ca, Mg), all 10 hamsters became pregnant and delivered 83 offsprings, their gender was 23 male (27.7%) and 60 female (72.3%) and in the third group, neither diabetic nor (Ca, Mg) all 10 hamsters became pregnant and delivered 90offsprings, 44 male (48.9%) and 46female (51.1%) (Table 2). The sex ratio of female to male in the first group of diabetic mothers (Ca, Mg) and in the second group, non diabetic (Ca, Mg) were 3.6:1-2.61:1, while this ratio in the third group, neither diabetic nor (Ca, Mg) was1.04:1respictively (Fig.1.2). The percentage of the female offspring of diabetic mothers (Ca, Mg) [78.57%] was higher than the female offspring in control group [51.1%] and also female offspring of non diabetic mothers (Ca, Mg) [72.3%] was higher than the female offspring in control group [51.1%] Fig.3.

The difference in the sex ratio between the first group diabetic mothers (Ca, Mg) and the second group non diabetic mothers (Ca, Mg) was not statistically significant (p-value – 0.99), while the difference between the group of diabetic mothers (Ca, Mg) with control group (p-value - 35.2) and between group non diabetic mothers (Ca, Mg) with control group (p-value -29.76) were statistically significant (Table 1). The Total no of offspring in the first group diabetic mothers (Ca, Mg) (70, 28.8%) was lower than the total numbers of offspring the second group, non diabetic (Ca, Mg) [83, 34.16%] and also in the third group, neither diabetic nor Ca, Mg [90, 37.4%] (Fig.4). Body weight in STZ-induced diabetes group increased from 160.15 g to180.15 g, while that of the control group increased from 160.15 g to 200.13 g 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 hamsters

Group	*Total no of offspring*	*No. of male offspring*	*% age of male offspring*	*No. of female offspring*	*% age female offspring*	*Sex ratio*
Diabetic (Ca, Mg)	70	15	21.43	55	78.57	3.6
Non diabetic (Ca, Mg)	83	23	27.7	60	72.3	2.61
Neither diabetic nor Ca, Mg	90	44	48.9	46	51.1	1.04

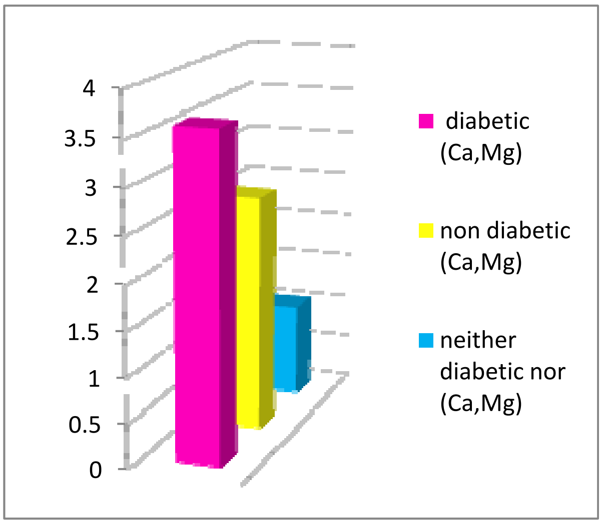

FIGURE 1. Male and female in different groups of hamsters

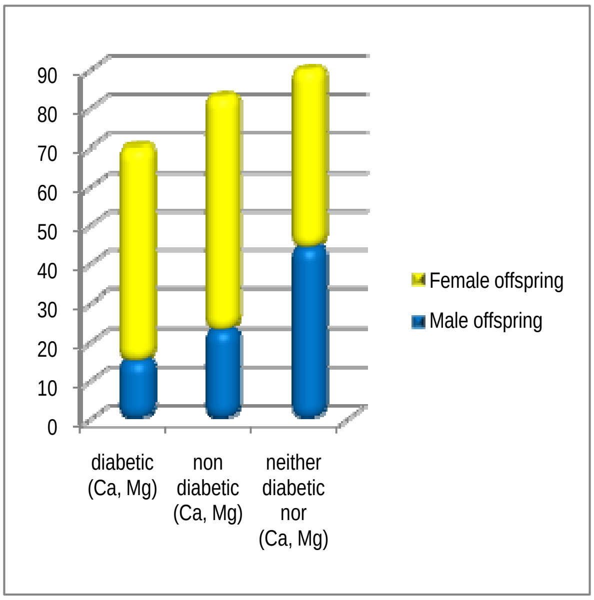

FIGURE 2. Male and female in different groups of hamsters

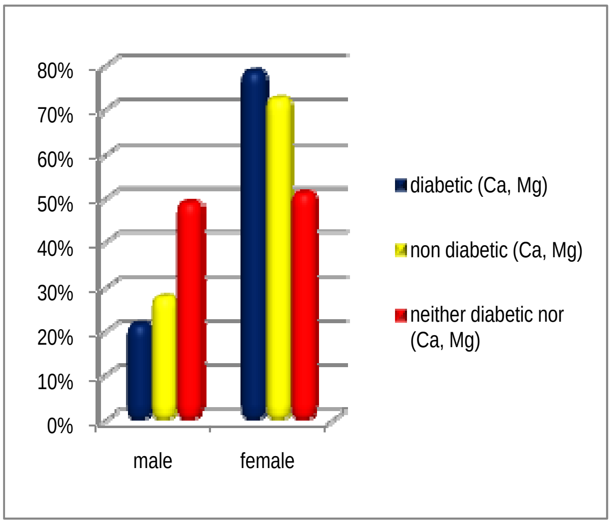

FIGURE 3. Offspring sex in different groups of hamsters

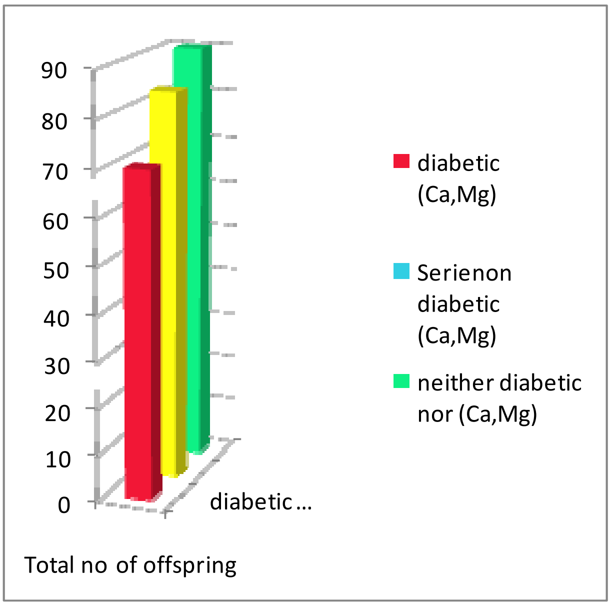

FIGURE 4. Number of offspring in different groups of hamsters

Streptozotocin has been widely used to induce type 1 diabetes in animal models especially rats and mice [13]. It has been reported that STZ induce dose-dependent diabetes administered either intravenously or intraperitoneally [15]. Intraperitoneal injection of STZ led to physiologic alterations consistent with reports of spontaneous and chemically induced diabetes in other animals [16, 17, 18]. Preselection 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 [19].

The first medical technique that can be used to select for sex is that of pre-natal diagnosis [PND] and abortion. To utilize this method, the pregnant woman must undergo some sort of prenatal testing, such as an amniocentesis, chorionic villus sampling or an ultrasound, which will allow the doctor to determine the sex of the child, among other things. Once the woman has the information about the child's sex, she can obtain an abortion if the fetus is not of the desired sex. The use of PND and abortion in order to select for sex sounds extreme, and indeed, as Edgar Dahl points out, it is not common for Westerners to utilize such a technique. For example, a follow-up study of 578 patients having prenatal diagnosis at one Melbourne centre found that none of the women had a termination because of the sex of the fetus .Going through the traumatizing experience of an abortion is usually seen as too high a price to pay for a child of a particular sex[20]

The second medical technique that can be used to select for sex is that of pre-implantation genetic diagnosis [PGD] with *in vitro* fertilization [IVF]. With this technique, the embryos are screened for sex prior to being implanted into the woman's uterus, thereby eliminating the need to later decide to terminate a pregnancy. PGD and IVF, however, are very invasive and potentially physically harmful, requiring the woman to go through at least one IVF cycle, which includes taking potent drugs to induce super-ovulation, extraction, fertilization and then testing and subsequent implantation of the embryos. Given the expense of IVF treatment cycles (according to IVF Canada in 2005 it cost $5,500 for one cycle of IVF, not including drugs[21] and, according to the same source, the drugs themselves can cost approximately $3,000 for one cycle)[22], it would be highly unlikely that it would be used as a technique for sex selection alone. More likely, it could be used as a sex selection technique for those who are already undergoing IVF for other medical reasons.

The last medical technique that can be used to select for sex is sperm sorting. New technologies allow sperm to be sorted into those carrying X or Y chromosomes with varying degrees of accuracy. To date, the most successful way in which to sort sperm is flow cytometry, which has been branded as the Micro Sort technique [21]. Sex selection using flow cytometry results from distinguishing between the identifiable differences between the X and Y chromosomes, as the X chromosome is larger than the Y. The sorted sperm is then used to artificially inseminate the woman. Studies have shown that the Micro Sort technique is more effective in selecting for girls, a success rate of 91%, than for boys, with a success rate of only 76%.12 Sperm sorting appears, then, to be the least invasive and least expensive (at about $2,300US per cycle13) method of selecting for sex.

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, savory foods leads to delivering a male and calcium rich foods to a female. 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 [24].

CONCLUSION

Today one of the good known methods on sex constitution is the preconception diet method. This method claims 80% accuracy and the theory is that by 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 parents 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. Langendon and Proctor first published 'the preconception Gender Diet `based on results reported [25]. The theory is that by altering 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 female, 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.

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 elevate relationship between minerals and sex ratio in hamsters. However, it is recommended to seek the advice of medical practitioner before going on such a restrictive diet, and stay on the diet for no longer than 3 months.

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