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The Effect of Different Concentrations of Calcium and Sodium on the Sperm Motility and Sex Determination in IVF Process

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Abstract

Objectives: This study aimed to investigate the effect of a treatment with sodium and calcium bicarbonate on the sex determination of a fetus.

Materials and Methods: A total of 60 mice were obtained from Pasteur Institute, Karaj, Iran. Female mice were stimulated to ovulate with PMSG hormone, and the oocyte was released by human chorionic gonadotropin (hCG) hormone. Sperms were collected from the male mice. In vitro fertilization (IVF) was performed in the completely sterile conditions in a control culture medium with pH=7.4. Then six experimental groups, three experimental groups with sodium bicarbonate as well as three experimental groups and the embryos, were transferred from the multicellular stage (the blastocyst) to pseudopregnant mice.

Results: Three groups treated with sodium bicarbonate with different pHs were significantly different from the control group regarding the percentage of the born male fetuses ($P \leq 0.05$); however, this difference was not significant between the sodium bicarbonate groups with different pH ($P \geq 0.05$). Among the groups receiving calcium bicarbonate treatment, only the average pH level of male infants at 7.7 demonstrated a significant distinction when compared to both the control group and the remaining treatment groups ($P \leq 0.05$).

Conclusions: Using sodium bicarbonate and calcium bicarbonate as well as adjusting the pH of the oocyte and sperm fertilization environment based on them may have been a safe and cheap method to determine the sex of a fetus.

Keywords: Calcium, Sodium, Sperm motility, Sex determination

Introduction

Several factors such as cultural, social, economic, and religious factors as well as medical issues such *sex*-linked inherited disorder have encouraged the couples to choose traditional or new treatment methods to choose their children's gender (1). In the livestock industry, moreover, the ability and possibility to determine the gender of the calf has long been pursued and is currently recognized as one of the most desirable reproduction techniques that can have major effects on the yield of milk and meat production (2). Recently, different laboratory methods have been developed to determine the sex of the child before pregnancy, including pre-implantation genetic diagnosis method, Ericsson or albumin gradient, microsort, flow cytometry, and pre-implantation genetic diagnosis, which is carried out after stimulation of ovulation by using intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) techniques (3, 4). Then, a blastomere is isolated from a 6-8 cell embryo by performing biopsy and the sex of the blastomere is determined by adopting fluorescence in situ hybridization (FISH) method or molecular methods; after the cultivation, an embryo with the desired gender is transferred to the uterus (5). The above method has high sensitivity; due to the high cost, complication, and low chance of pregnancy in this technique, however, researchers attempt to develop alternative methods. It has been argued that ions can affect the sex of the fetus (4). According to the available evidence, the presence of sodium ions in the diet, which affects the alkalinization of the uterine environment, increases the probability of having a male fetus (6,7). This may be due to the change in the tendency of the oocyte membrane to accept x,y sperms in different environmental conditions. According to the polarity cycle of the ovule membrane, the receptors of the ovule membrane accept or reject the spermatozoa containing x and y chromosomes selectively based on their energy cycle (8,9). This charge is not constant on

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Key Messages

- Using sodium bicarbonate and calcium bicarbonate as well as adjusting the pH of the oocyte and sperm fertilization environment based on them may have been a safe and cheap method to determine the sex of a fetus.

the ovule membrane, and it changes from positive to neutral or from neutral to negative in one cycle (10). Studies have shown that the polarity is predictable. When the oocyte membrane is negatively charged, it attracts spermatozoa containing the y chromosome – which has a positive charge, resulting in the male fetus development (11). Application of these materials can be considered as a natural approach to determining the gender. The above argument applies to the reason for the alkalinity of the culture medium containing spermatozoids in this technique (12). Therefore, the gender determination method based on the effect of different concentrations of calcium and sodium on the migration of spermatozoa can facilitate preventing the occurrence of sex-related genetic diseases that are passed from one generation to another. Given the above discussion, this study aimed to investigate the effect of a treatment with sodium and calcium bicarbonate on the sex determination of a fetus in IVF process as well as to examine the efficacy of IVF process.

Methods and Materials

Animals

A total of 60 mice were obtained from Pasteur Institute, Karaj, Iran and transferred to the lab. All animals were kept under the similar conditions, with a temperature of 18-22°C, humidity of 30-70%, as well as a period of 12 hours of lightness and 12 hours of darkness. They were provided with the required water and food which was obtained from the feed factory of the Pasteur Institute. In order to accustom the animals to the environment and remove the stress caused by the transportation, the animals were transferred to the lab one week before the initiation of any experiments.

Stimulation of Ovulation

Female mice were stimulated to ovulate with PMSG hormone, and the oocyte was released by the human chorionic gonadotropin (hCG) hormone. In sum, hCG hormone was injected 48 hours after PMSG hormone injection. Then 12-13 hours after the injection, the female mouse was dissected and the oviducts located after the ovaries were separated and placed in the dissection dish. Using an insulin syringe under the loop, the ampulla part of the oviduct was split and the ovules were released. As a result of the injection of hormones, the ampulla area was found to be bigger and clearer. The drop method was employed to wash the ovules. The ovules were removed from the ampulla area in masses and were completely surrounded by the cumulus. The oocytes were pipetted and put into the culture drop, which helped to separate the cumulus from the oocyte.

Sperm Collection Method

Male mice, whose fertility had been proven, were killed by cervical vertebrae displacement. After dissecting the tail of the epididymis, the vas deferens were first separated and several slices were made and placed in a test tube containing T6 culture medium with 15 mg/mL BSA and, then, were placed in an incubator for 45 minutes until the sperms were activated. A sample of the sperm was prepared and was observed by a light microscope. After observing the sperm sample under the microscope and confirming it in terms of the number, grade, and movement, an amount of it was transferred to the IVF drop by an oral pipette.

IVF Process

IVF drops contain T6 culture medium with 15 mg/mL BSA, and have a volume of 70 µL. They were placed in an incubator for 12 to 24 hours after instillation. To perform IVF in a completely sterile condition, the oocytes were extracted from the fallopian tube. Regarding the sperm samples that were pre-prepared in the control culture medium with a pH of 7.4, there were six experimental groups in total. Among these, three experimental groups received sodium bicarbonate treatment, while the remaining three experimental groups received different treatments (Figure 1).

Pseudopregnant Mice Creation for Embryo Transfer

First, male rats were vasectomy. Then 10 to 15 days after vasectomy of the male mice and when the sperms' exit from the sperm tube was completely confirmed, the adjacent to an adult unmated female mouse was performed. The body of the female mouse with a false pregnancy after the intercourse with a male was prepared to receive an embryo and, then, the resulting embryo was transferred to a pseudo-pregnant mouse. As for the embryo transfer, the mouse was anesthetized and the lower back area was fixed and sterilized with 70% alcohol. The embryos were transferred using the previously prepared Pasteur pipette. The embryos were then transferred from the multicellular stage (the blastocyst) to pseudopregnant mice. The blastocyst embryos were transferred to the uterine horns of pseudopregnant mice 2.5 and 3.5 days after mating.

Statistical Analysis

All data were statistically analyzed using SPSS software and performing Tukey and Duncan tests. The $P < 0.05$ was considered as a statistically significant level.

Results

As for IVF and embryo transfer, a 7- to 8-week-old female mice and an 8- to 12-week-old male mice whose fertility was confirmed in advance were used for vasectomy and IVF. In this study, nearly 950 eggs were obtained from

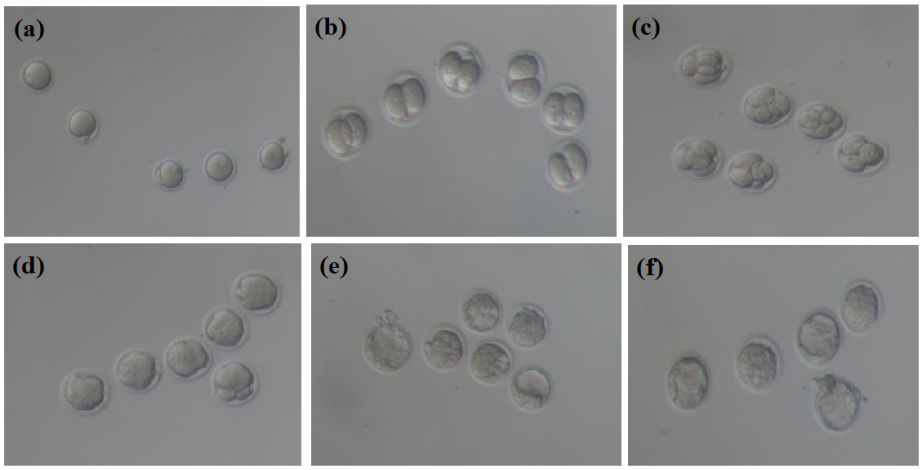

Figure 1. IVF Process (a) Oocyte, (b) 2-Cell Stage, (c) 4-cell stage, (d) Morula Stage, (e,f) Blastocyst Stage

50 mice for IVF, out of which 700 embryos were used for transfer after the fertilization and the remaining embryos were degenerated, infertile, and blocked. In the control group and in each experimental group, 10 pseudo-pregnant mice were considered for embryo transfer, and 10 embryos were transferred to each mouse.

Embryo Transfer Result

Out of 41 born fetuses in the control group (pH = 7.4), 20 ones were male. The percentage of male compared to females was 48.61%. In the experimental group 1 (pH = 7.5) whose pH was adjusted by sodium bicarbonate, 21 out of 33 born fetuses were male. The birth rate of male compared to female was 65.99%. In the experimental group 2 (pH = 7.6) whose pH was adjusted by sodium bicarbonate, 25 out of 40 born fetuses were male. The percentage of born male compared to female was 67.33%. Table 1 shows further details about experimental groups and the percentage of male and female.

The Effect of Sodium Bicarbonate on Percentage of Male Fetuses in Study Groups

Statistical analysis showed that the three groups treated with sodium bicarbonate with different PHs were significantly different from the control group in terms of the percentage of the born male fetuses ($P \leq 0.05$); however, this difference was not significant between the sodium bicarbonate groups with different PH ($P \geq 0.05$). Table 2 presents further details in this regard.

The Effect of Calcium Bicarbonate on Percentage of Male Fetuses in Study Groups.

The only significant difference observed in the treatment groups involving calcium bicarbonate was in the average pH level of male infants, specifically at 7.7. This pH level was notably distinct from both the control group and the other treatment groups ($P \leq 0.05$). More details on this regard are shown in Table 3.

Discussion

Sex determination has long been a distant dream for human (13). Some factors (e.g., the presence of some sex-related genetic diseases) have encouraged the couples to choose new treatment methods to determine their children's sex (14). Furthermore, having the option to

Table 1. The Total Number of Fetuses and Born Male Fetuses in the Studied Groups

Groups	Total fetus	Male fetus (%)
Control	41	20 (48.61)
Sodium bicarbonate (pH=7.5)	33	21 (65.99)
Sodium bicarbonate (pH=7.6)	40	25 (67.33)
Sodium bicarbonate (pH=7.7)	38	25 (68.49)
Calcium bicarbonate (pH=7.5)	47	17 (34.95)
Calcium bicarbonate (pH=7.6)	45	16 (34.35)
Calcium bicarbonate (pH=7.7)	30	10 (33.33)

Table 2. The Effect of Sodium Bicarbonate on Percentage of Male Fetuses in Study Groups

Groups	Male fetus (Mean± SD)	*P*
Control	48.61±3.27	$\leq 0.05^{A}$
Sodium bicarbonate (pH=7.5)	65.99±4.85	$\geq 0.05^{B}$
Sodium bicarbonate (pH=7.6)	67.33±3.63	$\geq 0.05^{B}$
Sodium bicarbonate (pH=7.7)	68.49±5.90	$\geq 0.05^{B}$

Note: *Similar letters indicate non-significance of the mean difference (≥0.05), and non-similar letters indicate the significant difference (≤0.05).

Table 3. The Effect of Calcium Bicarbonate on Percentage of Male Fetuses in Study Groups

Groups	Male fetus (Mean± SD)	*P*
Control	48.61±3.27	$\geq 0.05^{A}$
Calcium bicarbonate (pH=7.5)	34.95±4.65	$\geq 0.05^{A}$
Calcium bicarbonate (pH=7.6)	34.35±3.90	$\geq 0.05^{A}$
Calcium bicarbonate (pH=7.7)	33.66±3.11	$\leq 0.05^{B}$

Note: *Similar letters indicate non-significance of the mean difference (≥0.05), and non-similar letters indicate the significant difference (≤0.05).

choose the gender of children helps the parents establish a proper balance in the family. Although the ratio of boys and girls is equal in the societies, this ratio is not achieved in the families with children of the same gender, which may cause complex family problems. To address these problems, therefore, it seems necessary to develop effective methods aiming at improving the mental health of the society (15, 16). The sex determination technique can be also used in the livestock industry for improving the animal breeding, which is now considered as one of the most effective reproduction methods, and for fulfilling the human needs properly (17).

In the present study, the effects of the different concentrations of calcium and sodium on the migration of spermatozoa and the determination of the gender were investigated. To this end, seven groups were considered, including one control group and three experimental groups whose pHs were adjusted by sodium bicarbonate as well as three other experimental groups whose pHs were adjusted by calcium carbonate. It was found that the average birth of the male fetuses was significantly different from that of the female ones in the experimental groups where pH was adjusted by sodium bicarbonate (pH = 7.5, pH = 7.6 and pH = 7.7) compared to the control group with pH = 7.4. There is a hypothesis suggesting that very active sperms have y chromosome, because y sperms have more mobility due to their lower weight (18,19) . Two parameters, the rapid progressive motility and the normal morphology of sperm after separation operation based on the spermatozoid migration, can dramatically affect the pregnancy rate (20). Regarding the other three experimental groups whose pH was adjusted by calcium bicarbonate, the first group (pH=7.5) and the second group (pH=7.6) were not significantly different from the control group. However, the third group (pH=7.7) was significantly different from the third control group (P<0.05). In this alkaline environment, seemingly, the activity of spermatozoa containing Y chromosome decreased while the activity of spermatozoa containing X chromosome increased.

Evidence demonstrates that the presence of sodium ions in the diet, which affects the alkalinization of the uterine environment, increases the probability of being male. This may be due to the change in the tendency of the oocyte membrane to accept x,y sperms in different environmental conditions (21). According to the polarity cycle of the egg membrane, the receptors of the egg membrane accept or reject the spermatozoa containing x and y chromosomes selectively based on their energy cycle. This charge is not constant on the oocyte membrane, and it changes from positive to neutral or from neutral to negative in one cycle. According to the researchers, the polarity is predictable. When the oocyte membrane is negatively charged, it attracts spermatozoa containing the y chromosome with a positive charge, resulting in the male gender formation (22). Application of these materials can serve as a natural approach to determining the gender. The sodium ion probably affects the expression of genes such as SRY. The TDF sex-determining gene was identified in 1990 and was named SRY (23). However, other genes play roles in the regulation of this gene, and some factors such as pH, ions, and temperature can also influence the expression of these genes. The effects of some environmental factors (e.g., environmental temperature and pH) on the determination of the animals' sex have been confirmed, and this issue probably has an effect on gene expression; however, further studies are required to accurately evaluate these effects (24).

Marzec-Wróblewska et al investigated the effects of sodium, copper, and selenium levels on the human semen and glutathione peroxidase activity (GPx) in seminal plasma, and examined their relationships with sperm quality. The results of their study showed a significant relationship between sperm motility and selenium, as well as between GPx and rapid progressive motility. (25).

Previous methods used for determining the gender of a fetus have some disadvantages. For example, amniocentesis technique, in which a sample of amniotic fluid is removed from the mother's abdomen using a syringe containing cells of fetal origin, must be cultivated for diagnostic tests. This technique is one of the most widely used methods for prenatal diagnosis. The disadvantages of the amniocentesis technique are as follows: first, this method is invasive; second, the implantation may fail while performing it; and third, abortion is required when an abnormality is observed (26). In the method based on the effect of different concentrations of calcium and sodium on the migration of spermatozoa for determining the gender, it is possible to pick up the desired sperm and, therefore, to select the gender of the desired embryo. It is not necessary to initially create the embryo and, then, evaluate it genetically in order to determine the abnormalities. This technique is safe and does not require abortion. Furthermore, it is a non-invasive and successful method for determining the gender of the fetuses, which prevents the occurrence of sex-related genetic diseases and regulates the female-to-male ratio in, especially, Asian countries. Therefore, it can be employed as an effective method in this field to improve the mental health of the society. Also, finding out the gender of the fetus before implantation helps parents to determine their desired gender for implantation. Gender selection before implantation is now considered as an effective measure to prevent sex-related genetic diseases. Some opponents of the gender selection argue that it can prevent the birth of girls in different societies and cultures where females hold inferior positions. The ability to determine the sex has long been a desirable goal in the world's livestock industry, and now it is recognized as one of the most popular reproduction techniques that greatly contributes to the production of milk and meat, and can also be used to create and expand the livestock with a superior breed.

Conclusions

Using sodium and calcium bicarbonates as well as adjusting the pH of the oocyte and sperm fertilization environment through their application may have been a safe and cheap method to determine the sex of a fetus.

Authors' Contribution

AGH and NT was responsible for conceptualization and methodology, TM designed the study and led the conduction of the research. SK and FM took part in investigation and formal analysis of the results. PB contributed to validation and anlyses of the obtained results of the study. All authors contributed to writing-original draft preparation and review and editing. All authors approved the final manuscript and take responsibility for the integrity of the data.

Conflict of Interests

Authors have no conflict of interest.

Ethical Issues

The Ethics Committee of Avicenna Research Institute approved the study (Code: .REC.2015.62).

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