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Observational Study

Does gender selection works? An observational study

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Abstract

Sex selection is an option for couples who want to avoid passing a sex- linked genetic disease to their baby. For example, some females are carriers for over 350 X-linked diseases, even if the disease does not directly affect them. Hemophilia and Duchenne muscular dystrophy are examples of diseases that occur with a recessive gene on the X chromosome.

Some choose the sex of their baby because of a personal preference like "Family balancing." Many studies were done before to prefer one gender upon another.

When we look at the statistics the chances of having a boy or a girl are almost the same and there is no medical evidence to suggest we can influence

Design: Prospective randomized controlled study.

Setting: The study was conducted at LAMIS Clinic for Obstetrics & Gynecology from January 2015 to February 2022 and still continue up today.

The study started after observation that use of cyclofert female capsules before conceptions leads to more probability to male sex embryos.

Our study contains five thousand patients who received cyclofert female capsules at dose of two capsules per day for at least two months before conceptions. The data was compared with another one thousands patients who receive placebo. The two groups divided into three according to the age group. All patient included were on natural cycles and no ovulation inductions used, irrespective of the parity.

Objectives: To rule out that the observation which is noted that increase male gender embryos related to use of cyclofertfemal capsules or not.

Results: The overall pregnancy rate irrespective of the age was 86.02% (4301/5000) & 66.6% (666/1000) for the cyclofert group and the placebo group respectively. Overall abortion rate for cyclofert group was 1.4% (60/4301) while 9.3% (62/666) for placebo group. 89.69% (3804/4241) of the total pregnancy were male gender in cyclofert Group, while at placebo group it was 41.23 % (249/604), ($p<0.05$ statistically significant).

Conclusion: Gender selection could be work by non-invasive, not expensive, effective, affordable, and available without complication method by giving cyclofert female capsules, which is, increases the pregnancy rate, reduces the rate of abortion and increases the male gender embryos. Although, there is no guarantee that the baby will actually be one sex or the other, but cyclofertfemal capsules can influence what type of sperm cell will reach an ovum, and fertilize it. Therefore, yes, male gender selection may be technically possible.

Introduction

For decades, gender selection was a problem for many societies, this is because of multiple reasons., the probability of having a boy or a girl depends on whether the sperm that fertilizes the egg is X or Y. Sex selection is an option for couples who want to avoid passing a sex-linked genetic disease to their baby occur with a recessive gene on the X chromosome (Hemophilia and Duchenne muscular dystrophy [1]; Reduce the risk of their baby having a disease more common in certain sexes. For example, a family who has a child with autism spectrum disorder (ASD) might try for a girl because ASD has a higher male incidence, and their chances of having another affected boy is 25 percent. Alternatively, choose the sex of their baby because of a personal preference "family balancing" [2,3]. Today's sex-selection options aren't equally effective, affordable, or available. The most accurate sex-selection methods are the most expensive and often mean have to undergo invasive fertility treatments (IVF and PGT) [4-10]. This procedure requires to invest significant time and money, and often means have to take fertility drugs before the procedure with potential side effects. There are low-tech, inexpensive gender selection techniques as well. These range from the Shettles and

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Whelan methods to folk wisdom, The American Society for Reproductive Medicine (ASRM) says there's no evidence any of this can influence the sex of the baby. Many countries have taken legislative steps to reduce the incidence of sex-selective abortion. At the International Conference on Population and Development in 1994 over [11,12] 180 states agreed to eliminate "all forms of discrimination against the girl child and the root causes of son preference".

The World Health Organization and UNICEF, along with other United Nations agencies, have found that measures to reduce access to abortion are much less effective at reducing sex-selective abortions than measures to reduce gender inequality. Around 56 million abortions are performed each year in the world, with about 45% done unsafely. India has amongst the worst gender ratios in the world [11,12]. Recent studies have shown that the egg is not passive during the process of fertilization, we say also during sex determination.

Methods

Design

Prospective randomized controlled study.

Setting

The study was conducted at LAMIS clinic for Obstetrics & Gynecology. The period from January 2015 to Feberary 2022 and still continue up today.

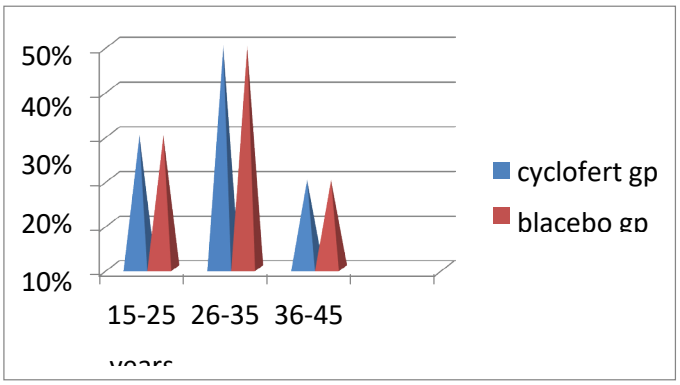
Our study contains five thousand patients who received cyclofert female capsuls at dose of two caps per day for at least two months before conceptions. The data where compared with another one thousands patients who receive placebo. The two groups divided into three according to the age group.

The patients undergoing for infertility treatment due to male factor, or assisted reproductive tech either IVF or AIH WHERE excluded, ectopic pregnancy excluded. Patient more than 46 years old were excluded too. All patient included were natural cycles and no ovulation inductions used irrespective of the parity.

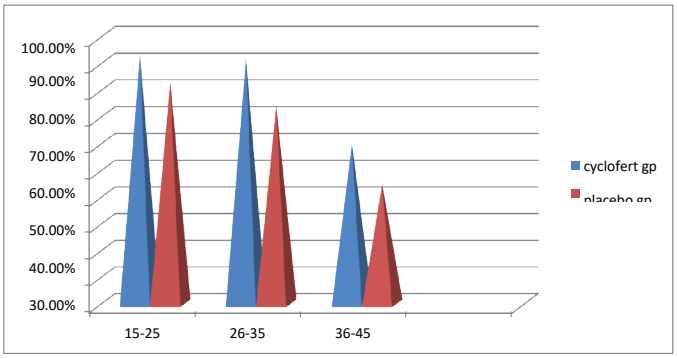
Results

Distribution of both groups according to age

Both cyclofert female group & placebo group are divided to three groups almost the same percentage.



Pregnancy rate during three cycles of both groups



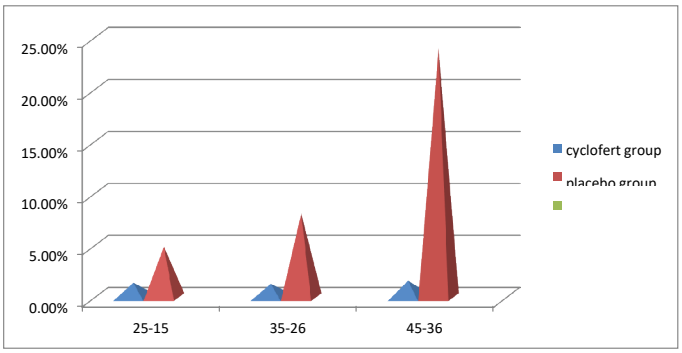
Pregnancy rate at 15-25 age group was (93.3% & 83.3%) at cyclofert group & placebo group respectively.

At 26-35 years age group was (92% & 74.2%) at cyclofert group & placebo group respectively

At 36-45 years age group was (60.2% & 45%) at cyclofert group & placebo group respectively

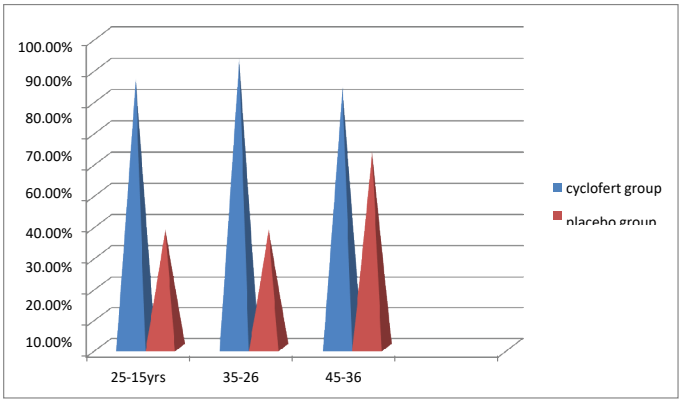
The overall pregnancy rate irrespective of the age was (86.02% & 66.6%) respectively.

Abortion rate at both groups & according to age group



Overall abortion rate at cyclofert group 1.4% while at placebo group 9.3% .(15-25 years age group 20 patients aborted at cyclofert group, while 10 patients aborted at placebo group) (1.4%,4.9%), at 26-35 years group 30 patients aborted at both groups (1.3%, 8.1%). At 36-45 years group 10 patients & 22 patients aborted (1.6% & 24%) respectively.

Gender outcome at each group according to the age group



89.69% (3804/4241) of the total pregnancy were male gender at cyclofert group, while at placebo group were 41.23% (249/604) ($p<0.05$ statistically significant).

Discussion

We have only touched on sex selection here, and it is a highly complex issue. There are a vast number of reasons people may prefer to have one sex to the other, and there are certain cultures that favor sons over daughters or daughters over sons [3]. Dubuc and Sivia [13] reported that sex selection is a controversial issue. The preference of sons and prenatal sex selection against females have resulted in significant imbalances in sex ratio at birth in several Asian countries, including India and China [11,12]. This issue becomes even more complicated when we consider our ideas of gender and the expectations we may have when choosing the sex of a child.

Many studies done before to prefer one gender upon another, Keep in mind that Mother Nature has already tipped the odds a bit in favor of boys [4-10,14,15,17-19].

According to data from the National Center for Health Statistics, approximately 105 boys are born for every 100 girls. There's always a 50% chance of conceiving a child of the sex wanted [13,19-21].

The probability of having a boy or a girl depends on whether the sperm that fertilizes the egg is X or Y bearing sperm The origin and maturation of both X and Y spermatozoa are the same, however, certain differences may exist [22-54].

Previous studies proposed a substantial difference between X and Y spermatozoa, however, recent studies (116) suggest negligible or no differences between these spermatozoa with respect to ratio, shape and size, motility and swimming pattern, strength, electric charge, pH, stress response, and aneuploidy. The only difference between X and Y spermatozoa lies in their DNA content [55-59]. Moreover, recent proteomic and genomic studies have identified a set of proteins and genes that are differentially expressed between X and Y spermatozoa.(Therefore, the difference in DNA content might be responsible for the differential expression of certain genes and proteins between these cells [60].

Preimplantation genetic testing (PGT)

A procedure can be done during IVF – after the eggs are fertilized and before an embryo is transferred, one or two cells are removed from an embryo and tested for genetic or chromosomal disorders and/or sex. There are two types of tests: PGT-A and PGT-M are 96

to 97 % accurate at determining the sex of the embryo [61,62] at our study the results show 89.69% of the total pregnancy were male gender at cyclofert group; i.e. almost good result. The PGD procedure is expensive, invasive, can be painful, Fertility drugs can have uncomfortable side effects including: weight gain, bloating, swelling, and blurred vision. Note that some fertility clinics offer preimplantation genetic testing only for medical reasons, and not for sex selection.

Ericsson method

This technique, named for its founder Ronald Ericsson, aims to separate faster-swimming, boy-producing sperm from slower- swimming, girl-producing sperm. It has used in combination with artificial insemination (AI). The technique claims to be 70 to 75% effective [63-69], with more favorable results for conceiving boys than girls. Some independent studies have supported these claims, while others have contested them. Inexpensive compared to higher-tech methods, noninvasive, relatively safe but there is no guarantee of success. Ericsson has published his own extensive research and claims a success rate of approximately 75 to 80 percent, but evaluations of the test have not been published by other fertility experts or proven independently and AI is not as effective as IVF, and it may take many cycles to achieve a pregnancy, depending on age and fertility.

MicroSort (Flow cytometry)

This technique involves staining the sperm with a fluorescent dye and then separating the male and female sperm cells using a flow cytometer. Once the sperm are identified, the preferred sperm can be placed in the uterus during ovulation using AI or IVF. In its initial clinical trial, MicroSort reported 90% effectiveness for female gender selection and 85 % for male gender selection [70-80]. However, these numbers have not been conclusively confirmed independently. There is some concern that techniques that modify sperm may affect sperm quality. Researchers continue to study this. To use MicroSort, couples must have at least one child and use the technique for the underrepresented gender or be a known carrier of a chromosomal-linked disorder. Note: MicroSort is a trademarked method that is not approved for use in the United States. In 2011, the FDA ordered MicroSort to stop clinical trials and stop using the process; It is still offered in Mexico, Switzerland, North Cyprus, Malaysia, Cambodia, Thailand, and Nigeria.

At-home gender selection techniques

Shettles method: Popular sex selection method .named after gynecologist Landrum Shettles. The method is based on a theory of sperm survival. The theory is that sperm bearing a Y chromosome move faster but do not live as long as sperm that carry X chromosomes 75% effective for choosing girls and 80% for choosing boys. In 1970, physician and researcher Landrum.

B. Shettles suggested that couples have intercourse on the day of ovulation or shortly after in order to produce a male offspring. The guidance is involved - even going into detail on who should orgasm first. The method also recommends certain sex positions, claiming that penetration from behind is the best way to conceive a baby boy. Does not require drugs or invasive medical procedure. Shettles explains his methods in a book titled Your Baby's Sex, Now You Can Chose. As of 2006, Shettles' book had six revised editions and sold over one million copies [81-85]

Whelan method: Couples should have intercourse two or three days prior to ovulation to increase their chances of conceiving a female infant. To increase the probability of conceiving a male infant, couples should have intercourse between four and six days before ovulation. According to Whelan's research, the method increases the probability of conceiving a male by eighty-six percent and increases the probability of conceiving a female by sixty-six percent. but many experts are doubtful. The Whelan method directly contradicts the Shettles method. The theory here is that the biochemical changes that may favor boy-producing sperm occur earlier in a woman's cycle. The Whelan method was different from the Shettles method. The Shettles method was developed seven years prior to the publication of Whelan's book. Whelan published her method in her book, Boy or Girl, in 1977. However, as part of her research, Whelan found that Shettles presented his method for use with natural conception, even though he based his method on studies about

artificial insemination. Whelan cited several studies in her book that demonstrated Shettles' method did not work. Since the publication of Whelan's book, many researchers have refuted her theories regarding the relationship between intercourse timing and an infant's sex. A study published in The New England Journal of Medicine in 1995 refuted all claims that intercourse timing affects the outcome of an infant's sex. That study asserted that there was no association between intercourse timing and sex outcome, There's no guarantee of success [86-90].

Gender selection kits: These at-home kits are based on the Shettles theory. Separate girl and boy kits include a thermometer, ovulation predictor test sticks, vitamins, herbal extracts, and douches that are supposedly intended to favor a specific sex. Kit makers claim a 96% success rate, but some medical experts say the manufacturer's claims have no scientific merit [26]. The success rate claimed by kit makers is questionable, Douching is not recommended, and can actually lead to other problems like vaginal infections [2].

The Chinese gender predictor chart: Legend has it the chart is more than 700 years old and was discovered in a royal tomb near Beijing. The technique involves converting the mother's age and the month of conception to dates on the Chinese lunar calendar, then cross-checking that data on a chart that predicts the baby's sex. The accuracy of the Chinese Gender Calendar Chart is questionable. Some say its accuracy is higher than other gender prediction tools. We've read that about 70% accurate. Others say it's 90% accurate. Researchers at the University of Michigan School of Public Health did a study to test the Chinese lunar calendar method of predicting a baby's sex. They reviewed the records of 2.8 million Swedish births. Then they used a website-customized algorithm to estimate each mother's lunar age and month of conception. When they checked the predictions of the Chinese baby calendar method against the sex of the children who were born, they concluded that the Chinese birth chart was correct about 50 percent of the time -- no more accurate than flipping a coin. There is no scientific basis for the Chinese Gender Calendar Chart, so the most we can guarantee is 50%.

What is the role of cyclofert in sex selection

In addition to the multivitamins and minerals, which contained in a cyclofert capsule needed for good ovum production; there are some natural components. Let's go and see these component and their effects on good ovulation, fertilization, implantation and hence boy sex selection. What factors influence these results, the probability of having a boy or a girl depends on whether the sperm that fertilizes the egg is X or Y bearing, so wondering here, why the cyclofert female capsule taken by the mother can influence whether the sperm that reaches the egg is X or Y, even though it is a random process.

The natural plants component which makes big difference

Licorice dry extract: Licorice is an herb that grows in parts of Europe and Asia. Licorice root contains glycyrrhizin, which can cause side effects when eaten in large amounts. The chemicals in licorice are thought to decrease swelling, decrease cough, and increase the chemicals in the body that heal ulcers. Licorice is used for eczema, swelling of the liver, mouth sores, and many other conditions; it was used traditionally for treating a variety of conditions, including lung, liver, circulatory, and kidney diseases. Today, licorice root is promoted as a dietary supplement for conditions such as digestive problems,

Table. Information about cyclofert female capsules

Composition	Per 1 Capsule
Maritime pine bark dry extract (pinusmaritima)	25 mg
Acetyl-carnitine	25 mg
Co-enzyme Q10	6.250 mg
Zinc	1.875 mg
Vitamin B6	0.75 mg
Vitamin B9 (Folic acid)	0.2 mg
Vitamin B12	0.375 µg
Yam Root dry extract	16 mg
Vitamin E	25 mg
Selenium	7 µg
Omega-3 fish oil TG12/50	225 mg
Licorice dry extract	7 mg

menopausal symptoms, cough, and bacterial and viral infections. Licorice Extract is useful to ease lungs congestion and coughing by helping to loosen thick mucus in airways, which makes cough more productive to expel & so as at the cervical mucus and, B. Shettles using a phase contrast microscope concluded that the small, round headed sperm contained male-producing Y chromosomes, while the large, oval- shaped sperm contained the female-producing X chromosomes [28]. Y sperm: are faster but survive for less time in the female genital tract [91]. X sperm: are slower, but they are more resistant and therefore survive longer. The mucolytic effect of Licorice dry extract facilitate the sperm movements. At the other hand, some researchers have found no morphological differences between human X sperm and Y sperm genotypes; and Y- sperm do not swim faster than X- sperm [92-98]. Scherker [3] reported that Y spermatozoa had a greater tolerance to alkaline pH than X spermatozoa and they moved faster in this media, whereas the reverse being true for acidic pH. To the best of our knowledge, this study among the recent studies that have discovered the role of vaginal pH in fetal sex prediction. It found that there was significant higher rate of male fetuses who were conceived by mothers with alkaline vaginal pH and also there was significant higher rate of female fetuses who were conceived by mothers with acidic vaginal pH.Recently, the study by Oyeyipo et al. [93] aimed to separate X-chromosome and Y-chromosome-bearing spermatozoa using methods based on the viability difference between the X-chromosome and Y-chromosome-bearing spermatozoa, The vaginal environment is generally acidic, while the cervix and uterus are generally alkaline. Licorice dry extract is intended to change the gastric medium to make it more alkaline. Hence, Licorice dry extract change the vaginal environment to make it more alkaline, favor y-bearing sperm to fertilize the egg. (For a boy, the woman's vaginal pH should be 7.5 to 9 [99-104].

Wild yam: Wild yam is a plant that has been promoted as natural DHEA because it contains diosgenin, which can be used in the lab to create estrogen and DHEA. People most commonly use wild yam as a "natural alterative" to estrogen therapy for symptoms of menopause, infertility, menstrual problems, and many other conditions [75,104] is believed to influence hormone balances in a way that can alleviate conditions like morning sickness, premenstrual syndrome , hot flashes, menstrual cramps, vaginal dryness, low libido, and osteoporosis. Herbal

supplements manufacturers will often describe wild yam as "natural estrogen" or "natural DHEA" .used for the commercial synthesis of cortisone, pregnenolone, progesterone, and other steroid products [105].

Capacitated sperm are attracted to progesterone, which is secreted from the cumulus cells surrounding the oocyte. [93,106-110] Wild yam, contains diosgenin needed for Synthesis of cortisone & required for progesterone synthesis. Progesterone binds to the CatSper receptor on the sperm membrane and increases intracellular calcium levels, causing hyperactive motility. The sperm will continue to swim towards higher concentrations of progesterone, effectively guiding it to the oocyte. (chemo attractants that activate and guide sperm to the oocyte [111].

Pine bark extract: They're evergreen trees that grow abundantly in many parts of the northern hemisphere and in some parts of the southern hemisphere as well. In the past, many cultures have used the bark, needles, resin, and nuts of pine trees as medicine [112]. In the 1940s, scientist Jacques Masquelier began studying the health effects of pine bark after learning that indigenous peoples of North America were using pine bark tea to heal scurvy and wounds [133]. It's thought that the extract's antioxidant, antimicrobial, and anti-inflammatory properties have the potential to improve conditions like cancer, heart disease, and neurodegenerative conditions like Alzheimer's disease [112]. Pine bark extract is particularly rich in plant compounds called polyphenols, which are likely responsible for its health-promoting benefits. itsconstituent antioxidative phenolics are potent neuroprotective agents that can maintain cell viability under oxidative stress [114].

The uterus aids in the steps of capacitation by secreting sterol binding albumin, lipoproteins, and protolithic and glycosidasic enzymes such as heparin, It's thought that the Pine bark extract's antioxidant, antimicrobial, and anti-inflammatory properties increase the uterine secretions [93,106,108-110,115]. The sperm acrosome reaction can be stimulated in vitro by substances a sperm cell may encounter naturally, such as progesterone or follicular fluid, as well as the more commonly used calcium ionophore [116,117]. As the sperm approaches the zonapellucida of the egg, which is necessary for initiating the acrosome reaction, the membrane surrounding the acrosome fuses with the plasma membrane of the sperm's head, exposing the contents of the acrosome. The contents include surface antigens necessary for binding to the egg's cell membrane, and numerous enzymes which are responsible for breaking through the egg's tough coating and allowing fertilization to occur [93,104,107,118]. When the sperm enters the vitelline space, receptors on the sperm head called Izumo1 bind to Juno on the oocyte membrane [91]. Once it is bound, two blocks to polyspermy then occur. After approximately 40 minutes, the other Juno receptors on the oocyte are lost from the membrane, causing it to no longer be fusogenic. The cortical reaction and acrosome reaction are both essential to ensure that only one sperm will fertilize an egg [23].

So the effect of cyclofert capsule in gender selection contains good ovum production, good ovulation, alkaline the vagina pH, thining of the cervical mucus, increase attractive power of the sperm towards the ovum and anti-oxidants, anti-inflammatory effect.

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