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RESEARCH ARTICLE

GENETICALLY MODIFIED SEXED SEMEN: A SIMPLE REVIEW ABOUT METHODOLOGY, APPLICATION AND FALLOUTS FOR PRESENT AND FUTURE GENERATIONS

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ABSTRACT

The field of animal reproduction especially that dealing with dairy sector, has seen the application of many innovative and sophisticated techniques in the recent past. Sexing semen is one such technology that has attracted the attention equally of dairy professionals and farmers as well. This is based on the principle of flow cytometry combined with the X and Y sperm separation techniques that are available in the domains of standard physics and biological sciences. The well known particle counting machines in fluid media like Coulter Counters are employed in this methodology not primarily to count the particles passing through the medium but instead charging them differentially and then to coordinate and guide their motion to respective electropositive and electronegative sides of such an elaborate set up which also includes equipments like micro jet throwers and laser lights; also involving chemical treatments of sperms with chemicals like fluorescent dyes. The particles in this technology are no doubt the sperm cells and the entire process is based on the assumption that a size difference does exist between X and Y sperms because X sperm have nearly 3.8% more DNA than the Y one. This difference is then utilized in a way to differentiate such sperms, X or Y and assign different charge, positive or negative to them and then direct them to different electric fields and hence separate them. Sperms are very delicate structures and are likely to have their DNA damaged or tempered and hence get genetically modified and impaired and obviously various repercussions of such methodologies are nothing but well known. This will certainly have ominous fallouts for present and future generations.

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INTRODUCTION

Dairying is a highly commercial segment these days. In many countries, this is run more or so, on the lines of industry. There are very big dairy farms which contain hundreds and even thousands of cattle. There is application of the latest and state of art technology in this profession. The financial as well as technological investment is very high. Dairy farmers of late have shown keen and growing interest in the application of latest know how and techniques which are meant to increase their return on money and make the profession more profiting. Male calf is seen more a nuisance and a hindrance on their profits. This generally increases bio-waste as more females mean more milk and increase in the number and size of herds. Moreover, the application of exotic breeding programs becomes more viable and efficient if the outcome is more female calves. So, a great and growing number of farmers have shown keen interest in the application of sexing techniques these days.

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The most common among them is the use of sexed semen which promises to produce more female calves than males. There is long history of the attempts to produce sexed semen (Ericsson, 1973; Gordon, 1958; Lindahl, 1956; Schroeder, 1939). There are numerous companies which market conventional semen to these farmers. Many of them have started to supply sexed semen as well. The deal appears attractive to farmers as well. Till, recently, everything seemed to go well and cozy but of late, growing evidence has started to appear of the instances of DNA of sexed sperm getting damaged. This damage on the DNA can certainly be of varying degree. It can be very less or even very severe. There is no test evolved so far which can tell for certainty that such and such sperm has a damaged DNA or not? Even if it is damaged, then damaged in which direction of genetic manipulation, there is no indication to show. There is very big and almost absolute consensus among the scientific community worldwide that genetic damage or tempering is a very big concern and a thing of great harm. The genetic modification in the DNA can play havoc with the natural genetic constitution of existing natural species of animals and plants and if it is happening in the very near and dear phylogenic relatives of human race and

especially in those animals which are an essential constituent of the normal food chain of humans; it is extremely dangerous. We all know that cattle are the biggest source of milk and meat which is eaten by humans. So, the matter becomes very sensitive and the danger of any genetic change or mutation creeping in these animals may find a route into manifestation in the human species; the chances can certainly not be ruled out. So, it becomes imperative to have an independent, unbiased and simple review on this important subject.

The mutagenic semen

There is no such thing as marketed in the world. The semen companies manufacture and market a product which is better known by the name "sexed semen". Everyone in the field including farmers and professionals are well aware with this name. Now it is a billion dollar question as of how a thing well marketed, labeled as sexed semen, becomes a mutagenic one; a term nobody is even slightly aware of as existing. The answer is not in the domain of general knowledge but on the contrary, it is hidden very deep in the scientific truth of the entire methodology and technique of its manufacture. There should be no eyebrows raised, if there is fairly good sexed semen, which anyone can vouch for that it is totally free of genetic damage or modification but it is not so in the real case scenario. Any dose of sexed semen may contain the genetically tempered and defective DNA and it may also be true that none such dose may contain any such deformity. Nobody can say for certain that such and such a dose is hundred percent free of the genetic damage. The villain in the story is not the equipments or agents that are utilized in the manufacture and marketing of such a thing called sexed semen but the processes, mechanisms and working of scientific phenomena that are involved in its making. Science appears to play the villain but we all know that science is the biggest friend of man. So, it becomes imperative that we go a little deep in scientific reasoning and understanding to unearth the mystery behind the existence and manufacture of mutagenic semen.

Unintentional mutation

The irony of the fact is that even the companies involved in its production and marketing do not primarily intend to formulate it as a mutagenic variety but the laws of science and biology in particular provide enough basis where all this gets happened just of its own and not by intention. Sexed semen is converted into mutagenic semen by processes that are not in the control or choice of anyone whosoever, company or persons who want to produce it, may be for the benefit of society and this is the thing that is more dangerous in this entire episode and should draw the immediate attention of world society with utmost urgency. There are of course huge profits involved in all this and profit is the most pious word in the community of business and when a multinational business corporation tastes profit it is just like tasting blood by a member of some carnivorous species. This is here that it becomes a different question whether these companies are already in knowledge of the fact that they are marketing a genetic poison in the name of sexed semen or not?

Even if they know it and want to cover up for this attribute of it and they are successful in it too, they can certainly go about in propagating this business further and further. But if they know it for certain, then it is their foremost duty to stop this or at least inform the public of this fact. It is an altogether different fact if they really are not into this knowledge but the fact is that why should the entire humanity and future generations of it suffer from the fact of the ignorance or greed or both of these giant business corporations?

The problem is that the present state of affairs in this matter has already plunged the very genetic existence of human race in danger. The points are very simple and need a deliberate discussion and we will try to touch them in a very brief and simple manner. Let's spare few moments only to appraise ourselves of the impending danger on the very genetic survival that we are so proud to claim as humans as the superior most creation of all genetic evolution.

The mutagenic process

The problem starts to emerge from this point only that it is certainly not a bad idea to raise milk and meat productions in the world but the malady lies in the way this new technology works. The main principles involved in sperm sexing as described by George Seidel (How are sperm sexed? SAVMA Symposium, 2002) are as follows:

1. Bovine X chromosome has 3.8% more DNA than Y chromosome.
2. The dye Hoechst 33342 binds to DNA quantitatively i.e. the more DNA, the more dye that binds.
3. When a certain wavelength of light is beamed at Hoechst 33342 bound to sperm, the dye fluoresces, giving off intense blue light. So, the sperm with X chromosome give off 3.8% more light.
4. The flow cytometer/sperm sorter has all the components needed to accomplish the steps just described, including a laser to provide the correct wavelength of light to excite the dye, a detector to measure the amount of light and a computer to analyze the information.
5. There are also additional components like the sperm are pumped through a vibrator tube that breaks the streams of exciting fluid into small droplets that contain sperms. Droplets containing X sperm are given a positive electrical charge and deflected into a collector tube and the droplets containing Y sperm are given negative charge and deflected into a different collector tube. Dead sperm and those that cannot be sexed are not collected.

So, the sperm sexing procedure is a set of steps. The sperms exit the equipment at a speed of 60 miles/hour and are collected in tubes containing fluid to cushion that fall. After collection, the sperm are frozen, thawed and sent for insemination. This is the basic technology and it has remained by and large same during all these years. The problem lies somewhere in the way this technology works and very interesting facts have come to light in the course of further research in this field. The sperm are very delicate micro entities covered not even by a strong cell wall but by a very thin and soft plasmalemma (Hafez, 1982). They are not even complete cells. They are haploid structures (Hunter, 1982) whereas a complete cell has to be a diploid body in these species. So, they cannot withstand harsh treatment of speeds up to 60 miles/ hour, throwing into ultra thin streams and then into micro droplets, getting charged and struck with laser beams. They get damaged. This is a fact as put by Funston and Meyer (2012) by saying that "process of sorting does damage sperm and reduces fertility when compared with conventional

sperm". Also as George Seidel (2014) also described, "this lowered fertility, in part due to damage to sperm during sorting". The weak and delicate sperms get damaged and there is no ground to believe that their DNA is not damaged even then and any damage or alteration in DNA is a potential mutation or at least if some of these alterations get combined into one, they can cause a big mutation to happen in the very next or forthcoming generation. DNA is an elaborate molecular structure and also the genetic code of plant or animal species and any structural changes in it are bound to lead to genetic changes in the next generations. As Palma GA *et al* (2008) has confirmed "ultrastructural alterations in blastocysts produced from sex sorted sperm". Further if some chemicals like the dye that is used to stain the DNA also penetrates deep into the DNA molecules, may cause chemical changes in the DNA molecular bands. This dye may be Hoechst 33342 which is mainly used or any other one but the fact is that it has to penetrate deep inside otherwise it will not be able to produce fluorescence that is so required to differentiate between X and Y chromosome bearing sperms. Any deep molecular encounter of outer chemicals with the DNA may be of a dye; can lead to structural alterations in the DNA molecule. This is another danger (Garner, 2009) that "Another possible cause might be the side effects of Hoechst 33342 retained in spermatozoa that is used to label DNA to enable differentiation of X or Y chromosome bearing spermatozoa during sorting. There is evidence that Hoechst 33342 is transmitted into the oocyte by the fertilizing sex sorted sperm and is detectable in the cytoplasm of resultant embryo even until 8-celled stage". The damaged or altered DNA of the embryo is certainly not the normal one. It is changed, deteriorated or spoiled one but it is definitely not the one that is decided by nature after millions of years of the natural evolution of species. So, any outer, unwanted and unnatural change is a direct intrusion into the normal genetic existence and essence of living species. This may become fatal also and if carried on forcibly for a long period over many generations may lead to the origin of some unwanted traits in the concerned species or even it may provide enough ground for a new species to emerge. It may be a big statement to make but certainly such possibility cannot be ruled out.

The first indications of the reaction of nature's forces appear at this stage. The resultant embryos are rejected by the laws of life perfected by nature. The embryos are killed and aborted by way of developmental arrest. As Telford *et al* (1990) has describes as "It seems evident that developmental arrest of embryos caused by sperm sorting occurred following the cleavage stage, presumably during the transition from maternal to embryonic genomic control that occurs at the 8-celled stage in bovines". This happens because the defected sperm can also get a chance to fertilize the egg (Henkel R et al, 2004) as described, "spermatozoa with damaged DNA can indeed fertilize the oocyte". Such damage may be species specific or animal specific (Inaba *et al.,* 2016). He has put it "reduced blastocyst development was associated with X sorted sperm in one bull". He had four bulls in his experiment and even one bull in four makes it 25% of populations of bulls involved and this is a quite big and dangerous ratio. The truth of above discussion is that genetic changes in DNA of the concerned species do happen due to the process of sex sorting and this fact is proved beyond doubt from the above discussion. But there is one more dimension to this danger that is explained in the below paragraph. The knowledge we have about such tempering in DNA is about a limited span extending to the stages of pre-embryonic developments only and mainly the studies have remained focused around the blastula and cleavage stages of zygote. But the most subtle truth that everybody should know is that these changes may get manifested even in later stages of development of not only of the embryo but also of the fetus too. There have started to pour in reports of comparative greater mortality of calves delivered from the sexed sperm as compared to the conventional semen. This clearly proves that the mutation has definitely crept in there and it makes these sexed calves more vulnerable to forces of death than the ordinary ones. The laws of genetics have a still more dangerous story to be revealed right from the days of great Gregor Mendel. We all know of the recessive genes. They were called as 'alleles' by Mendel. They are known to exist and here are the ominous bells ready to ring. If by chance, such a mutation has taken place by the act of sexing sperm and it lies dormant as a recessive mutation for a generation or two and then one fine day it expresses itself as the dominant gene after many a generations and it is powerful and devastating too; what the thing then we will be left with to do? The answer is a big desperation. Can we let the humanity in a lurch in the wait for such a time bomb catastrophe to happen? The rightful answer should be the assertive and plausible 'no'.

A danger too big and real than the GM crops

There is no questioning that the world has definitely entered a threat stage where the danger of genetic poisoning in humans and animals is very, very real. This danger is far more damaging than the danger of genetically modified (GM) crops posing a perceptible threat to genetic constitution of various animal species including humans which feed on these crops for food or fodder. The picture with sexed semen is that it produces mutation in the species of animals on which it is applied by the direct and first step only if the particular sperm in it has gotten genetically tempered. It is like injecting mutation into the zygote because once the genetically altered sperm fuses with the normal ovum, the resultant zygote and embryo has to be a mutant one and there is no escaping this fact. It does not take the long course of GM crops where an animal or man may eat such a genetically altered crop or its fruit and the mutation will enter the living system of that animal or man by a very long and until a limit even dubious route of getting into the somatic system of the organism concerned. This is a fact well known these days (Beal et al, 2017; Goldman and Shields, 2003; Murad, 2017) that such chemicals, agents or materials do exist that upon eating can cause a damage in the genetic tissues of man or animals and such changes can be inherited. Still the world has not forgotten the thalidomide disaster (Kim and Scialli, 2011). But in the case of GM crops the intermediate steps also include very complicated and exhaustive processes involved in the entire digestive process and the chances may be that only some metabolites, molecules or enzymes will make it through into the blood stream of the animal concerned and all the genetically altered tissues of such a crop are passed out of the body of such an animal or man as such as excreta or feces. The probability of such a genetical modification to pass into the genetic tissue of the animal concerned is remote. But here in case of sexed semen which has already become mutagenic and the horrible truth is that we even do not know of this fact; it produces mutation directly in the genetic tissue of the offspring as such as the sperm fuses with the ovum of the female animal and mutation is directly transmitted through the fertilization process. The process is very direct, simple and quick. There

are no long or intermediate steps in between. There is not even the chance of the carriage of such a mutation through a somatic route with which there is a probability factor whether it will happen or. The genetically modified crop only interacts with the somatic cells and tissues mainly of the digestive tract of the animal concerned. Even if it is able to induce a mutation there, the chances are that such a mutation will not be transmitted to the next generations of that animal because in order to getting this mutation transmitted in next generations, it has to pass into the germplasm of the animal concerned which is a very rare possibility in such a situation (of course, hundred percent it cannot be ruled out) because there are very different mechanisms of the behavior and multiplication of germinal and somatic cells and a differentiation between the two is always maintained by the laws of existence of life and nature. But in case of mutagenic sperm, the picture is entirely different as such as a change has already gotten manifested in the germ cells only. Hence the situation here is not only alarming but is catastrophic too.

Big lobbies, publicity and propaganda

It is interesting to note that sex sorted semen is known as sexed semen which produces sexed progenies and it has found a widespread usage in the dairy sector worldwide. Along with big multinational corporations behind this trade, there are various governments and big lobbies involved into the implementation, publicity and propaganda of very high proportions that has blinded almost every critical eye that can raise even slightest of a doubt over the possible dangers and side effects of this technology. The picture which is presented to public until this point looks very attractive and rosy that a new technology has emerged in the world which produces progenies of desired sex in cattle and also it can find use in other animal species like camel, horses, goats, pigs, sheep etc and even in humans. This can lead to a stage where we can selectively have male or female meat and milch animals in greater numbers and this can usher in an era of plentifulness of dairy and beef products and starving humanity especially in poor nations can be fed and nourished in a much better way by the application of this technology on a very large scale. This propaganda is already going in full swing in almost all the countries of the world and sexed semen companies are producing millions of doses of it annually and selling to governments, corporations and lobbies. Due to this even the farmers who get tempted to give a new technology a chance to increase the profits of their dairy farms; are prompted to buy this and all this is going on a very large scale from many years in a row.

Directionless and unquantified mutation is more dangerous

The directionless mutation is far more dangerous than the engineered one for in case of an engineered mutation that we see in case of the development of GM crops, we already know that the given mutation will happen in what direction of genetic manifestation and even we know its approximate magnitude but in case of the directionless and unintended mutations, we do not at all know about the upcoming mutations of either their nature or of their magnitude. Such mutations certainly may be far more devastating than expected. One more point is that we will not even be able to detect them and their faulty effects and genes will go on passing generation after generation until the point when they become super devastating to a level that it will definitely be impossible to undo the harmful effects of them. If a cow produces milk that contains mutagenic properties or our children feed upon the beef that has mutant elements in it, we probably will not be able to perceive these effects in generation one or two and in future generations when these defective genes will accumulate in greater numbers and we will be encountering a generation of humans who might have developed taste buds, canines and digestive habits like the carnivorous animals and who will prefer to feast on raw human flesh like living Draculas; then it will be too late in repenting over the crime of present day multinational corporations marketing sexed semen to our existing generation. There will be no point in crying after billions and billions of tons of water might have flown down the Thames, Ganges, Amazon, Niles or Volga by then.

Conclusion

Genetics is the basis of life and genetic code of every living species is the essence of its existence. Any threat that poses a danger to the natural existence of species should be done away with. If such a danger starts to lurk over humans or a species that is very close to humans phyllogenically or in behavior, existence or social interaction; then a very serious note should be taken of it and efforts should be concentrated to eliminate such a danger. So, any further activity and decision on the use or not of sexed semen should be decided after a thorough and comprehensive review on this subject by world scientific community and by the national as well as international leadership of nations.

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