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An Exercise into Sex Fixing of Progenies in Dairy Animals and Calculating the Impact Factor of Such a Drug Discovery with Reference to Indian Conditions

B.S. Aulakh

Department of Applied Pharmacology, Gregor Mendel Institute for Research in Genetics, No. 144/2, Netaji Park, Baloke Road, Haibowal Kalan, Ludhiana, INDIA

Corresponding author: BS Aulakh; Email: bsaulakhpost@rediffmail.com

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ABSTRACT

Sex fixing of mammalian progenies is one of the most fascinating subjects ever reported and it can be a formidable tool in increasing dairy productivity as well as ensuring considerable economic and social benefits. There have been many recorded attempts to produce sexed off-springs in mammals and most of them involved semen sexing methodologies. The present methodology involves a novel in vivo technology duly patented and which uses the administration of a liquid dosage form which has active ingredients like monosodium ethanoate and ethanoic acid which when given orally to females of dairy animals; yields female off-springs with impressive success rate. A huge trial of this methodology was conducted in real field conditions in Gujarat and the outcome was impressive.

Keywords: Sex, fixing, progenies, manipulation, ratios

Mammals are the most dominant animals on earth today. So, the production of sexed offsprings in them has marked economic and social implications. Many scientists have worked on this since the first half of the last century. There were mainly three lines on which such research was conducted. First were the attempts to alter the –pH of female genital tract. Second were the attempts to produce sexed semen (a sexed semen is the one containing the accentuated proportions of either X or Y bearing spermatozoa) and the third were the use of certain chemicals, hormones, sera etc to produce a shift in the sex ratio. Recently much emphasis is laid on the attempts to understand the exact mechanism of sperm-egg fusion. Candidate molecules have been identified that act as receptors on the ZP (zona pellucida) or the ovum membrane and bind with possible sperm ligands. Along with this the differential in vitro binding of X and Y containing sperms with different antigen-antibody media based on the assumption of differential binding sites on them and their successive elutriation in accentuated proportions has gained attraction. The present research also known as Aulprofem technique (Kebede *et al.,* 2013), is an *in vivo* attempt to produce female offsprings where sex ratios are manipulated probably by binding of receptor sites for Y sperm on the ovum by the interaction of certain moieties that are generated in the living system by the action of the active constituents in the oral medication which is given to the female animals prior to insemination. This leaves greater scope and chances for binding of X sperm with the ovum as receptor sites for it are left unbound and open. Among various outcomes of this discovery may be a rapid increase in the numbers of females of high yielding milch varieties of cows and buffaloes. This will definitely be taking the profession of dairying to new heights by increasing milk production alongside benefiting dairy industry too.

MATERIALS AND METHODS

The present exercise was an impressive field trial conducted jointly by J.K. Trust-Gram Vikas Yojana, Ahmedabad and Ansh Pharma, a private company at Vadodara which owned the marketing rights of this

technique under the co-ordination and technical support from Gregor Mendel Institute for Research in Genetics, Ludhiana under the guidance and vigil of B.S. Aulakh, the discoverer and patent holder of this technology (Aulakh, 2008) who also happens to be the founder and present director of this institute as well as the author of this article. The methodology involved was an in vivo technology duly disclosed and described as patented in USA. It is in the form of a liquid oral dosage form which is administered to the female animals; cows and buffaloes, just before mating or AI (artificial insemination). It was procured from Ansh Pharma as conveniently packed in unit doses of 225 ml each in PET bottles, labeled as Aulprofem and with instructions to be stored in moderate temperature conditions of 8 ºC to 25 ºC in a cool dry place. The active constituents of this medication formula consisted of as having 0.6 gm of monosodium ethanoate dissolved in 10% v/v ethanoic acid q.s. to make required solution to 10 ml of such a preparation. It is declared that no animals were harmed or injured in any way during such an exercise. The statutory approval by the institutional ethical committee was taken. It may further be added that the above active constituents are classified in all major pharmacopeias of the world namely, US, British, Australian and Indian etc, and also in Martindale Drug Pharmacopoeia; as pharmaceutical aids. Hence they are naturally as belonging to the category of most safe and harmless compounds. So, the question of animals getting harmed or affected in any way does not arise during the course of such an exercise. It was a huge exercise covering 194 animals in total spreading through 3 districts of Gujarat province namely Jamnagar, Surendranagar and Vadodara. The data was recorded as based on the administration of the drug in actual field conditions and care for various factors such as abortions, stillbirths, progress of pregnancy etc were regularly and routinely taken and checked and results of the sex of the off-springs were noted on actual deliveries. The inferences were drawn on simple percentage base and various outcomes and impact factors calculated in simple methodology.

RESULTS AND DISCUSSION

Study was initially planned to be covering about 300 animals but in actuality it covered 194 animals which is again an impressive figure in this type of experimentation. There were 128 conceptions and luckily, all animals successfully delivered of which 23 were male and 105 female calves. The results are as depicted in table 1.

Table 1: The results on cows and buffaloes subjected to manipulation of sex ratios

Sl. No	No. of Inseminations	No. of pregnancies	No. of animals died, abortions/ Miscarriages etc.	Male Offsprings	Female Offsprings
1	194	128	00	23	105

On simple calculation, we have with us 82.03% female calves. This means that male calves are mere 17.97%. This seems to convey that an imaginary unitary sex ratio at birth of 50:50; males to females is manipulated in such a way that it has moved up on the percentage scale about 32 points. This means that sex ratio is manipulated a mere 32% from the original imaginary sex ratio of 50% females to the now manipulated sex ratio of 82%. But actually this is not so because now the original male sex ratio at 50% has also moved down to a value of 18%. So, now we have 82 females at birth for every 18 males. In the present calculation of 105 female births for 23 male births, this turns out to be 456 female calves at birth for every 100 male calves born. In another words, now the manipulated sex ratio is 456%. These results can be better understood with the help of table 2 where it is well elaborated by taking the examples of sex ratio manipulations on female side from 60% to 80% on the simple percentage scale.

One more fact should be taken into consideration that secondary sex ratios are seldom unitary. In most of the cases in dairy animals, they are male dominated i.e. there are more male births than female ones. So, if a sex ratio in a sex fixer exercise is manipulated on the female side, this gives more value and performance weightage to such a technique. In the present exercise of sex fixing, we have 23 male calves whereas the number of female calves is 105. This means an excess of 82 female calves at the end of the sex fixing exercise. Had there been no such exercise being undertaken, we could have gotten 64 female as well as male calves both. Now we have 105 female calves. This means that we have 41 more female calves produced as the visible boon of the sex fixer drug. Calculating from an Indian context because this exercise was undertaken in India, we can safely conclude that the given technology of

Table 2: Showing the actual increase in number of female calves per a hundred of male calves produced as a result of the use of sex fixer drug

Sl. No.	Imaginary unitary sex ratio at birth per 100 calves born, before the trial; males: females	Manipulated sex ratio at birth per 100 calves born, after the trial; males: females	Percentage increase in sex ratio per 100 calves born that appears on percentage scale, after the trial	Manipulated sex ratio as per number of male: female calves born, per every 100 male calves born; after the trial
1	50:50	40:60	10%	100:150
2	50:50	30:70	20%	100:233
3	50:50	20:80	30%	100:400
4	50:50	18:82	32%	100:456

Table 3: Showing the increased national benefit to farmers due to the use of sex fixer drug

Sl. No.	No. of animals taken for application of sex fixer drug used in present study	Immediate national benefit to the farmers as the value of additional female calves in Indian rupees in billions	Cost of the sex fixer drug valued at a unit price of approx. rupees 500; in Indian rupees in billions	Further national benefit to the farmers as the value of fully grown animals in Indian rupees in billions	Annual national benefit to the farmers from the value of milk produced in Indian rupees in billions
1	194	0.00615	0.00097	0.00216	0.0054
2	100, 000	0.317	0.05	1.113	2.783
3	10, 000, 000	31.7	5	111. 3	278.3
4	100, 000, 000	317	50	1113	2783

this sex fixer drug discovery has already gifted the farmers of Gujarat province in India, an additional 41 female calves. Since such a valuable technology is usually tried in animals of superior and valuable exotic breeds, so it derives that we have now additional 41 valuable calves of better exotic breed stuff. If each of these calves is fixed a conservative price value of Indian rupees 15,000; this means that farmers of Gujarat who benefited from this trial have already pocketed a sum of nearly rupees 6,15,000. So, even if such a technology is priced at around a unit price of rupees 500 each meaning an input cost of nearly rupees 97, 000 because 194 units were used in the entire exercise; this has generated an additional national profit of almost rupees 5,18, 000 in the first step only. One more aspect of this research is that these additional 41 calves that have emerged from a beforehand figure of nowhere are now a valuable national asset. If about 20% of them i.e. 8 of them die of calf mortality and more 20% of the remaining ones i.e. about 6 of them succumb to death before reaching maturity thus leaving us with a figure of 27 fully grown and mature, additional superior breed cows and buffaloes ready to deliver. If each one of them is priced at a mere rupees 80, 000; this means that Indian farmers have further pocketed rupees, 21, 60, 000. Further if each one of these of arguably exotic breed cows and buffaloes yields a conservative milk yield of 4000 liters per lactation cycle. This means that at a price of rupees 50 per liter of milk this becomes an output per animal of nearly rupees 2,00, 000 per a lactation cycle. Again this means an additional national revenue for such 27 animals at nearly rupees 5, 400, 000. This is a huge figure of nearly 5.4 million rupees. Suppose this cycle of lactations goes on year after year for many years, this means an annual contribution of equal amount per year if the lactation cycle is of about one year each. Even if it is a little more or less, it does not make much a difference as rupees 5.4 millions is a very, very huge amount as compared to the input cost just explained above of the sex fixer drug standing at about rupees 97, 000. The corresponding figures of the cost of drug if such a trial sample is extended to animals; hundred thousand, ten millions and hundred millions in numbers, stands at values of 50 millions, five billions and fifty billions in Indian rupees but the outcome increase in national annual income of farmers comes out to be at

exorbitant figures of 2.783 billions, 278.3 billions and 2783 billions as depicted in table 3.

Further, such benefits as the immediate benefit as the price of the female calves born and the value of fully grown animals for same numbers of animals taken for trial are also given in the same table. It may also be added that India has an animal wealth of nearly 300 million cows and buffaloes of which nearly 160 millions are of breedable category. The value of Indian rupees at present is at about 65 rupees per a US dollar. To correlate this calculation with respect to economies of other nations, this exchange rate value of Indian rupee should be kept in mind.

Most of the animals that received the treatment during this sex fixer exercise, delivered female offsprings. The success rate could have been more if this was a further small and closely organized and coordinated clinical trial. Because this was a large and field trial, the chances of erroneous handling are usually more in field conditions in this type of trials. The failures can be attributed to manual error in handling, spillage, storage and transport in adverse conditions and faulty dose adjustments in case of heavier animals. Sex fixing in mammals has a long history. Many scientists have worked on this (Beernik and Ericsson, 1982; Bhattacharya *et al.,* 1966; Corson *et al*., 1984; Ericsson, 1973; Gledhill, 1983; Gordon, 1958; Lindahl, 1956; Sampson *et al.,* 1983 Schroeder, 1939). The major drawbacks of these techniques remained the lack of laboratory tests to evaluate the degree of sperm separation (Hafez, 1982) and the inability to know the precise mechanism of binding and fusion of mammalian sperm with ova. Overall, the manipulation of mammalian sex ratio has still remained a mirage on the horizon (Hunter, 1982). The fusion of a sperm with ovum is the most magnificent event of the world but our knowledge on this important aspect of life is still limited. Recently there have been attempts to study the effects of certain compounds like tetraspanins like CD9 (Hemler, 2003), CD81 (Cormier *et al*., 2004), glycosylphosphatidylinositol anchored proteins like CD55 (Coonrad *et al*., 1999), integrins (He *et al*., 2003), disintegrins (Primakoff *et al*., 2000), fertilins, cyritestin (Cho *et al*., 1998) etc on the process of gamete fusion. These researches have clearly established the presence of receptor sites on oocyte and their respective ligands on the surface of sperms. But the bigger questions are far from being answered. The 'tetraspanin-web' is still a big mystery (Boucheix *et al*., 2001). The role of other candidate molecules the likes of which are mentioned above; needs a lot more investigating. Recently there have been attempts to differentially bind X and Y bearing spermatozoa using H-Y antigen antibody interactions using non protein substrata such as agarose beads and U.S. patents granted for them (Bryant 1984; Zavos and Dawson, 1991). These developments clearly demonstrate the existence of differential binding sites for X and Y sperms and this difference can be exploited in developing a viable technology for sex fixing in mammals, either in vivo or in vitro. The present technology is an in vivo technique and it can be hypothesized that the material of present research produces certain YSBLM (Y sperm binding ligand mimics) moieties in the living system and also in the genital system of the female animals undergoing this treatment in such a way that these YSBLM moieties differentially bind with candidate receptor sites on oocyte involving ZP and membrane binding and penetration to inhibit their binding ability and/or their fusion with candidate ligands on the Y bearing sperm. The importance of this technology becomes even more marked with the view that it will not only provide an insight into the differential behaving and working of X and Y bearing sperms in the process of fertilization but also provide valuable data and knowledge on the role played by the candidate molecules in understanding fully well the exact mechanism of gamete fusion.

CONCLUSION

It is well evident from the outcome of this sex fixing trial that sex fixer drugs are effective in altering the mammalian sex ratios and a new class of drugs known as sex fixers has emerged and more and more of sex fixer drugs will be discovered in future.

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