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RESEARCH ARTICLE

A BRIEF ANALYSIS AND IMPACT FACTOR CALCULATION ABOUT A SEX FIXING EXERCISE IN DAIRY ANIMALS UNDERTAKEN IN NORTH INDIAN TERRAINS LIKE PUNJAB

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ABSTRACT

Sex fixing of mammalian progenies is one of the most fascinating subjects ever reported and if properly developed and put to application value, 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 in a variety of countries world over including USA, Canada, Australia etc and which uses the administration of a dosage form which has active ingredients like monosodium ethanoate and ethanoic acid in solution form which when given orally a little while before insemination to females of dairy animals, cows and buffaloes; yields female off-springs with impressive success rate. A huge trial of this methodology was conducted in real field conditions in Punjab spread over a vast area covering nearly ten districts involving variations in temperature and climatic habitat conditions and the outcome was impressive. The analysis and impact factor calculation of such a discovery were undertaken to assess the application value of such a drug discovery with reference to Indian conditions especially with a view to North Indian terrains like Punjab.

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INTRODUCTION

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.

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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. The same methodology has yielded excellent results (Aulakh, 2018) in various field conditions in India and is already a success story of profound dimensions. 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 a massive field trial conducted jointly by Department of Animal Husbandry, Government of Punjab, India and Rudra Meditech Pvt Ltd, a private company 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 Rudra Meditech 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°C to 25°C 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 1034 animals in total spreading through 10 districts of Punjab province namely Amritsar, Ludhiana, Moga, Patiala, Fatehgarh Sahib, Mohali, Gurdaspur, Ropar, Jullundhur and Sangrur with ten doctors each from first six of them and five doctors each from last four ones. Overall, 80 doctors took part in the trial. The trial was undertaken in real field conditions and data as issued in document no. 6/58/08/D-4/22076, by the Department of Animal Husbandry, Punjab; was recorded as based in actual working conditions and various factors such as abortions, stillbirths, progress of pregnancy etc were regularly and routinely checked and 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 1200 animals but in actuality it covered 1034 animals which is again a huge figure in this type of experimentation. There were 608 conceptions and 574 successful and healthy deliveries of which 168 were male and 406 female calves. The results are as depicted in Table no. 1. On simple calculation, we have with us 70.73% female calves. This means that male calves are mere 29.27%. 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 21 points. This means that sex ratio is manipulated a mere 21% from the original imaginary sex ratio of 50% females to the now manipulated sex ratio of 71%. But actually this is not so because now the original male sex ratio at 50% has also moved down to a value of 29%. So, now we have 71 females at birth for every 29 males. In the present calculation of 406 female births for 168 male births, this turns out to be 242 female calves at birth for every 100 male calves born. In another words, now the manipulated sex ratio is 242%. These results can be better understood with the help of Table no. 2 where it is

well elaborated by taking the examples of sex ratio manipulations on female side from 60% to 71.73% 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 168 male calves whereas the number of female calves is 406. This means an excess of 238 female calves at the end of the sex fixing exercise. Had there been no such exercise being undertaken, we could have gotten 287 female as well as male calves both. Now we have 406 female calves. This means that we have 119 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 this sex fixer drug discovery has already gifted the farmers of Punjab province in India, an additional 119 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 119 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 Punjab who benefited from this trial have already pocketed a sum of nearly rupees 17, 85,000. So, even if such a technology is priced at around a unit price of rupees 500 each meaning an input cost of nearly rupees 5,00, 000 because 1034 units were used in the entire exercise; this has generated an additional national profit of almost rupees 12, 85, 000 in the first step only.

One more aspect of this research is that these additional 119 calves that have emerged from a beforehand figure of nowhere are now a valuable national asset. If about 20% of them i.e. 24 of them die of calf mortality and more 20% of the remaining ones i.e. 19 of them succumb to death before reaching maturity thus leaving us with a figure of 76 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, 60, 80, 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 76 animals at nearly rupees 15, 200, 000. This is a huge figure of nearly 15.2 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 15.2 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 half million. 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 1.52 billions, 152 billions and 1520 billions as depicted in table no. 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.

Table 1. The results on cows & buffaloes subjected to manipulation of sex ratios

Sr. No	No. of Inseminations	No. of pregnancies	No. of animals died, abortions/Miscarriages etc	Male Offsprings	Female Offsprings
1.	1034	608	34	168	406

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

Sr. No.	Sex ratio at birth, males: females before the trial	Sex ratio at birth, males: females, after the trial	Sex ratio increase on female side as depicted on a simple percentage scale, after the trial	Sex ratio as per actual number of male: female calves born, after the trial
1.	50:50	40:60	10%	100:150
2.	50:50	30:70	20%	100:233
3.	50:50	29.27:70.73	20.73%	100:242

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

Sr. 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.	1000(approx)	0.001285	0.0005	0.00608	0.0152
2.	100, 000	0.1285	0.05	0.608	1.52
3.	10, 000, 000	12.85	5	60.8	152
4.	100, 000, 000	128.5	50	608	1520

Table 4: Showing the impact of present drug discovery on the percentage increase in the national annual GDP of India

Sr. No.	Combined figure for the total value of the price of mature animals and the total value of the milk produced in their first lactation cycle in Indian rupees in billions	National GDP of India in Indian rupees in billions	Percentage increase in the national annual GDP due to the application of drug
1.	2,128	1,60,000	1.33

Table 5: Showing the impact of present drug discovery on the percentage increase in national annual GDP of India on repeat usage for five consecutive years

Sr No.	Figure for GDP increase from first batch of animals in Indian rupees in billions	Figure for GDP increase from second batch of animals in Indian rupees in billions	Figure for GDP increase from third batch of animals in Indian rupees in billions	Figure for GDP increase from fourth batch of animals in Indian rupees in billions	Figure for GDP increase from fifth batch of animals in Indian rupees in billions	Total national annual GDP increase in Indian rupees in billions	Percentage increase in national GDP calculated with base year with total GDP of 1,60,000 billions in Indian rupees
1 st year of productivity	2,128	Nil	Nil	Nil	Nil	2,128	1.33
2 nd year of productivity	1,915	2,128	Nil	Nil	Nil	4,043	2.53
3 rd year of productivity	1,723	1,915	2,128	Nil	Nil	5,766	3.60
4 th year of productivity	1,465	1,723	1,915	2,128	Nil	7,231	4.52
5 th year of productivity	1,245	1,465	1,723	1,915	2,128	8,476	5.30

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 small and closely organized and coordinated clinical trial. Because this was a huge 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 (zona pellucida) and membrane binding and penetration to inhibit their binding ability and/or their fusion with candidate ligands on the Y bearing sperm. Such examples have already started to pour in scientific history as described by Aulakh (2018) in another study. 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 bond molecules in understanding fully well the exact mechanism of gamete fusion.

Impact factor calculation

As depicted in table no. 3, the agricultural and economic output of such an exercise on national scale is simply tremendous. The combined figures for the value of fully grown animals which now are no doubt valuable national assets and the value of the increased milk yield also are the figures for the increase in annual national GDP (gross domestic product) in economic terms. GDP by definition is the monetary value of all goods and services produced within the geographic borders of a country within a specified period of time. At present the annual GDP of India is approx. 2.5 trillion US dollars.

In Indian rupees, this is something about 1, 60,000 billions. If such a technology is applied on animals; hundred millions in numbers in India, the relevant figures for increase in annual national GDP of India are shown in table no. 4. If such a technology is applied on national level in same magnitude continuously for merely five years in a row and we assume that the first batch of animals subjected to the application of this drug become mature and deliver between age; 3-4 years per animal, this means that at the end of a maximum of 4 years after the start of the technology on the first batch of animals, we start to reap the fruits of the benefits of this technology which are nothing but the total price value of the additional mature female animals who have already delivered their first offsprings and the value of the additional milk produced by them as a visible boon of this technology. So, all this gets happened at the end of 4-5 years from the start of the application of this technology. We can call this year as the 1st year of productivity. Similarly the results of the drug applied on similar magnitude in the second batch of animals will be available at the end of 5-6 years from the start of the exercise and conveniently this year can also be called as the 2nd year of productivity. Suppose the entire exercise extends to five continuous years in a row, then the results of the last batch of application will materialize at the end of 8-9 years after the start of such an operation and this year will be called as the 5th year of productivity. There is another angle to it.

The female animals that are born as the result of the application of the drug on the first batch of animals and yield national outputs in the form of the value of mature female animals who have delivered and the value of milk produced by them in their first lactations, continue to yield such values in their next lactation cycles also in the next year or so. So, in the next year when we will have outcomes in the form of benefits from the second batch of animals, we will also have benefits from the first batch too. Suppose, nearly ten percent of animals from the first batch succumb to mortality or go dry or whatever and there is approximately ten percent decrease in the national output from such animals in the form of the value of animals and the value of milk produced both. Then in the present case given the national GDP of India stands at nearly 1, 60, 000 billions in Indian rupees, the contribution from such animals in national yield will be at a value of 1,915 billions rupees i.e. ten percent less than the relevant contribution in the previous year as depicted in table no. 5. If more ten percent decrease happens in the next year, then the relevant figure will be rupees 1,723 billions. If further decrease in such values happens @ 15% per year for two successive years; the corresponding values will be 1,465 and 1,245 billions in Indian rupees.. The similar figures will pour in for other batches of animals as well. Now we will be left with an interesting picture if we view such figures as given in Table no. 5, for five consecutive years when first year figures from last fifth year batch will pour. This will coincide with the fifth year figures from the first batch of animals plus the fourth year figures from the second batch of animals plus the third year figures from the third batch of animals and plus the second year figures as well from the fourth batch of animals.

The corresponding figures for the national annual increase in Indian GDP are also given in this table. In the 5th year of productivity, the increase in national annual GDP of India will be approx. 5.33 percent and this is a huge figure. So, any drug discovery that is so capable of causing such a revolutionary upswing in the national income as well as in GDP of a country will be sufficient enough to start an entirely new chapter in ensuing in an era of bountiful prosperity in any country of the world which has an important element of dairy and agriculture in its national economy like India. The social as well as economic impact factor of such a research is simply beyond imagination. So, it derives from above discussion that sex fixer drugs are going to play very important role in the societal as well as national lives of people of various countries in a great way and they are here to stay and rightfully they will proclaim a status as an independent class of drugs. We, for convenience sake will learn to know them as sex fixer drugs. The same success story can very simply be emulated in any country of the world and in any geographical area with dairy and agrarian background and similar impact factor can be found to be very easily materialized.

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