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RESEARCH ARTICLE

A BRIEF DESCRIPTION AND IMPACT FACTOR CALCULATION ABOUT A SEX FIXING EXERCISE IN DAIRY ANIMALS UNDERTAKEN IN INDIA

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ABSTRACT

Sex fixing of mammalian progenies has been a cherished dream of reproductive physiologists since long and it can be a formidable tool in increasing dairy productivity alongside ensuring considerable economic and social benefits. Many attempts to produce sexed off springs have been reported in scientific history and most of them involved techniques producing sexed semen. The present methodology is a novel in vivo technology which uses the administration of a liquid oral formulation which contains active ingredients like monosodiumethanoate and ethanoic acid which when given to females of dairy animals; cows and buffaloes before insemination, yields female off-springs with considerable success rate. The given technology is very simple, effective and has already been patented in USA and many countries of the world including Canada, New Zealand, Australia etc. Major advantages of this may be a rapid increase in the numbers of females of high yielding milch varieties of cows and buffaloes thereby ensuring increased milk production along with benefiting dairy industry too. With so many benefits in its basket of success, this new methodology is even strong enough a candidate to proclaim the arrival of a new class of drugs known as sex fixers in the already diverse and magnificent world of drugs.

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INTRODUCTION

Mammals are the most important family of animals on earth today. So there are great social and economic benefits of the production of sexed offsprings in them. Many researches have worked on this since the first half of the last century. Firstly, there were attempts to alter the –pH of the female genital tract. Secondly, there were efforts to produce sexed semen (sexed semen is the one containing accentuated proportions of either X or Y bearing spermatozoa) and the third were the application of certain chemicals, hormones, sera etc to produce a shift in the sex ratio. Also, these days much emphasis has been focused on the need to understand the exact mechanism of sperm-egg fusion. Certain compounds have been identified that act as receptors on the ZP (zona pellucida) or the ovum membrane and bind with possible sperm ligands.

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Also, the differential in vitro binding of X and Y containing sperms with different antigen-antibody media has been reported which is based on the theory of existence of differential binding sites on them and their successive elutriation in accentuated proportions. 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 altered probably by binding of receptor sites for Y sperm on the ovum by the interaction of certain moieties that are generated in the body system by the action of the chemical constituents in the oral medication which is given to the female animals prior to insemination. This leaves relatively more 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. The important benefits of this may include rapid increase in milk production as well as having greater social and economic benefits.

MATERIALS AND METHODS

The present exercise was a massive field trial conducted jointly by the Commissionerate of Animal Husbandry, Government of Tamil Nadu, India and Rudra Meditech Pvt Ltd, a private company which owned the legal proprietary rights of this technique under the co-ordination and technical support from Gregor Mendel Institute for Research in Genetics, Ludhiana under the guidance and supervision of B.S. Aulakh, the discoverer and patent holder of this technology 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 (Aulakh, 2008). 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 8ºC to 25ºC in a cool and dry place. The chemical constituents of this drug formula consisted of as having 0.6 gm of monosodiumethanoate dissolved in 10% v/v ethanoic acid q.s. to make the 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 chemical 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 an impressive exercise covering 177 animals in the districts of Kanchivaram and Vellore. The data was recorded and issued as document no 46921/HI/09 by the Commisionerate of Animal Husbandry and was based on the administration of the drug in actual field conditions and various factors such as abortions, stillbirths, progress of pregnancy etc were regularly and routinely checked and results of the sex of the off-springs were noted on actual deliveries. The inferences were drawn on simple percentage basis and various outcomes and impact factors calculated in simple methodology.

RESULTS AND DISCUSSION

Study was initially planned to be covering 200 animals but in actuality it covered 177 animals which is again an impressive figure in this type of experimentation. There were 113 conceptions and 94 successful and healthy deliveries of which 19 were male and 75 female calves (Table 1). One more aspect of this trial is revealed in the manner in which secondary sex ratio i.e. sex ratio at birth, is reported as altered in this operation. We have with us 80% female calves. This means that male calves are mere 20%. 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 a mere about 30 points. This gives a weak impression that the already existent percentage of females at 50% has moved just about 30 points more to a percentage of about 80%.

This literally leaves us to draw the conclusion that sex ratio increase on the female side has been manipulated a nearly 60% i.e. from the already existent 50% to the nearly about 80% at the end of the sex fixing exercise. But in reality it is not so. The 30% pull up on the female side of sex ratio, automatically pushes the resultant sex ratio on male side down 30 points i.e. to a value of 20% from the one existent at 50% at the start of the exercise. Now we have a strange derivation that at a stage well before the sex fixing exercise, we had 50 female births for every 50 male births but at the end of the sex fixing exercise, we have 80 females for every 20 males at birth. This means an increase of 60 females in excess of male calves. In other words this means that we have 400% more females i.e. 400 females for every 100 male births. Had there been no sex fixing exercise undertaken; we could have satisfied ourselves at having a mere 100 female calves whereas now at the end of such an exercise, we have about 300 female calves in excess of the male ones. 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. This can also be summed up as saying that in a total of 500 deliveries; we have now 400 female calves whereas in a unitary sex ratio at birth, we could have gotten 250 female calves as well as 250 male calves.

This also is an increase of 150 female calves. Had there been no sex fixing exercise being undertaken, we could not have the additional benefit of having these 150 more female calves. One more fact should be taken into consideration that secondary sex ratios (sex ratios at birth) are seldom unitary in mammals. 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 because it is certainly comparatively more difficult to move a male dominant sex ratio at birth to the one which is more female dominant. In the present exercise of sex fixing, we have 19 male calves whereas the number of female calves is 75. This means an excess of 56 female calves at the end of the sex fixing exercise. Had there been no such exercise being undertaken, we could have gotten 47 female as well as male calves both. Now we have 75 female calves. This means that we have 28 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 Tamil Nadu province in India, an additional 28 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 28 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 Tamil Nadu who benefited from this trial have already pocketed a sum of nearly rupees 4, 20,000. So, even if such a technology is priced at around a unit price of rupees 500 each meaning an input cost of nearly rupees 88, 500 because 177 units were used in the entire exercise; this has generated an additional national profit of almost rupees 3, 31, 500 in the first step only. One more aspect of this research is that these additional 28 calves that have emerged from a beforehand figure of nowhere are now a valuable national asset. If about 20% of them i.e. about 6 of them die of calf mortality and

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.	177	113	19	19	75

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	40%	100:233
3.	50:50	20:80	60%	100:400

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.	177	0.003315	0.000885	0.0144	0.0036
2.	100, 000	0.18728	0.05	0.8135	2.0339
3.	10, 000, 000	18.728	5	81.35	203.39
4.	100, 000, 000	187.280	50	813.5	2033.9

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	Combined figure of total value of previous column in US dollars calculated @ rupees 65 a US dollar in billions	National GDP of India in US dollars in billions	Percentage increase in the national annual GDP due to the application of drug
1.	2847.4	43.8	2500	1.75

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 US dollars in billions	Figure for GDP increase from second batch of animals in US dollars in billions	Figure for GDP increase from third batch of animals in US dollars in billions	Figure for GDP increase from fourth batch of animals in US dollars in billions	Figure for GDP increase from fifth batch of animals in US dollars in billions	Total national annual Indian GDP increase in US dollars in billions	Percentage increase in national annual GDP calculated with base year with total GDP of 2.5 trillion US dollars
1st year of productivity	43.80	Nil	Nil	Nil	Nil	43.8	1.75
2nd year of productivity	39.42	43.8	Nil	Nil	Nil	83.22	3.33
3rd year of productivity	35.48	39.42	43.8	Nil	Nil	118.70	4.75
4th year of productivity	30.16	35.48	39.42	43.8	Nil	148.86	5.95
5th year of productivity	25.64	30.16	35.48	39.42	43.8	174.50	6.98

more 20% of the remaining ones i.e. about 4 of them succumb to death before reaching maturity thus leaving us with a figure of 18 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, 14, 40, 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 18 animals at nearly rupees 3, 600, 000. This is a huge figure of nearly 3.6 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 3.6 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 88,500. 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 2.0339 billions (i.e. very close to 2 billions), 200 billions

and 2000 billions in Indian rupees 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 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 at 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. 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 produce) 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. 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, al 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 2.5 trillion US dollars, the contribution from such animals in national yield will be at a value of 39.42 billion US dollars 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 dollars US 35.48 billions. If further decrease in such values happens @ 15% per year for two successive years; the corresponding values will be 30.16 and 25.64 billions in US dollars.

If the price of Indian rupee falls or increases considerably from the given value of rupees 65 a US dollar, then the size of national GDP as well as such share of this given technology will also fluctuate accordingly but by and large the resultant percentage effect of it on national GDP will remain the same because the change in exchange rate will pull the overall size of national GDP and the contribution of this drug technology in a similar way up or down. 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 5^{th} year of productivity, the increase in national annual GDP of India will be approx. seven percent and this is a huge figure. So, any drug discovery that is so capable of causing such a 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.

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