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Current Status of Sex Sorted Semen and its Long Term effect on Population Dynamics and Y-Chromosome Degeneration of the Breed Among Dairy Animals in Jharkhand, India: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Sex Sorted Semen gives the liberty of producing offspring of the desired sex - in farming animals by using it in conjunction with other assisted reproductive technologies such as Artificial Insemination and In-Vitro Fertilization after selecting the healthy sperm and separating into X-Female and Y-male Chromosome bearing populations based on their DNA content. It is an important biotechnological tool to increase the milk production and the profitability of Dairy Industry. Current study deals with the Principle, methods, main method, advantages, disadvantages and the current status of Sex sorted semen in India and Jharkhand. The main emphasis of this study is to draw the attention of Scientific fraternity towards the effect of Sex Sorted Semen on Population dynamics. The Sex Sorted semen increases the deviation of ratio between Male and Female Population from ideal 1:1, thereby decreasing the effective population size Ne and thus slowly reducing the viability and

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survivability of the population or breed concerned. Further the already depleting Y chromosomes will be reaped off all its genes in long run at a faster rate due to antagonistic selection pressure arising out of Artificial selection via Sex Sorted Semen acting against all the gene of Y chromosome of the breed or population concerned which might disturb many vital genes and the associated functions. The degeneration and extinction of scientists have been predicted long ago. The effect of Sex Sorted semen on Y- chromosome degeneration is yet to be pointed out, calculated and subsequently verified in any of the literatures. SSS is indeed a boon for India as well as Jharkhand. It might be too early to predict about the negative impact of SSS on population dynamics and Y-Chromosome degeneration. Further research work must be done to assess the extent and authencity of above mentioned impact(Predicted theoretically) by calculation as well as practical field based Experimentation.

Keywords: Flow cytometry; DNA; autosomes; selection intensity; Y chromosomes; population dynamics; Ne.

1. INTRODUCTION

India is unique in its appreciation of the cow culturally with less than 40% of India's cows productive. This coupled with high milk demand renders the male calf not productive and thus non-required and are often castrated early in life and mostly often unattended [1] So In reference to today's context of dairy industry, in order to gain competitive edge in the market, producers need to develop suitable strategies and Sexed Semen is one of them. Taking advantage of sexed semen technology, heifers will be born as often as 95% of the time, instead of 49% of the time when using semen that is not sorted for sex [1]

According to data of NDDB, the total bovine population of india was 299.6 million out of Adult female bovine population was 133.3 millions.(Livestock Census, Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture & Farmers' Welfare, GoI, www.nddb.coop). To increase the number of female bovine species, Sex sorted semen is no less than a boon. Bovine population of Jharkhand is constituted of only Non-Descript Cattles and Buffalo. So breed upgradation programmes coupled with A.I. with sorted semen would hugely help to increase the productivity of milk and to strengthen the dairy sector in Jharkhand. India has average milk production of about 176.3 million tonnes during the year 2017-18 with per capita availability of 375 gms/day. (Basic Animal Husbandry Statistics, DAHD&F, GoI).The associated use of reproductive biotechnology and genetic improvements combined with adequate sanitary and nutritional management are essential conditions for sustainable intensified animal production and financial autonomy within farms as the demand for livestock products like meat, milk and dairy products is increasing day by day globally, to meet this demand, among many emerging technologies for the above mentioned cause, sperm sexing or sexed semen is one of the newest reproductive technologies available to livestock sector [2]

The Semen Having X or Y bearing sperms and the capacity to produce a progeny of desired sex either male or female with (80-90)% accuracy are called as sexed semen. It was developed in Beltsvile, Maryland by United States Department of Agriculture (USDA), researchers in Livermore, California and Beltsville Maryland and patented as "Beltsville Sperm Sexing Technology" .Brahma Genetics Facility is the first and only bovine semen sexing lab in India. ABS India, in 2017, deployed Genus IntelliGenTM technology at Brahma, near Pune in Maharashtra.With IntelliGenTM, ABS India is producing and providing sexed semen for Holstein, Jersey, Sahiwal, Gir, Red Sindhi, Crossbreeds and Murrah and Mehsana buffaloes for the first time in India under the brand SEXCELTM (https://genusabsindia.com) .

Literatures are in plenty with advantages and disadvantages of Sex Sorted Semen in context of India. Current review deals with holistic study of Sex Sorted Semen (SSS) in India together with long term effect of Sex Sorted on Population Dynamics and degeneration of Y chromosomes and effect of artificial selection via SSS on Holandric genes Hitherto undiscussed.

2. DIFFERENCE BETWEEN X AND Y CHROMOSOME

As is clear from researches that mammalian sex chromosomes are dimorphic. This difference is used as the basis for sex sorting. The difference is being tabulated broadly as below in Table 1.

Table 1. Difference between x and y chromosome

FACTORS	X CHROMOSOME	Y CHROMOSOME
DNA	MORE	LESS
SIZE	LARGER	SMALLER
MOTILITY	SLOWER	FASTER
SURFACE CHARGE	NEGATIVE	POSITIVE
HY ANTIGEN	ABSENT	PRESENT

3. PRINCIPAL OF SPERM SORTING BY DIFFERENT METHODS

There are different methods of sperm sorting based on one or the other difference between X and Y chromosomes.They are being enlisted below as Table No. 2. Out of all the methods enlisted, Flow cytometry is the best.

4. FLOW CYTOMETRY

In case of flow cytometry, the autosomes carried by X and Y spermatozoa have identical DNA content and the difference in DNA mass of the X and Y chromosome has formed the basis upon which flow cytometric analysis of DNA in X and Y spermatozoa has been carried out. One of the first reports describing the analysis of sperm DNA using flow cytometry was made by Gledhill [15] It was followed by ability to distinguish mouse X and Y using Flow Cytometry. The difference in DNA content of X and Y forms the basis of flow cytometric analysis of DNA content in X and Y [2,16] The difference in DNA content in domestic animals between X and Y chromosomes bearing spermatozoa ranges from (3-4.5)% [17] According to Kumar et.al. [18], Flow Cytometry uses Flourescent dye that stains DNA fluorescent [19,20] It uses Flourescent Dye that stains the DNA OF the spermatozoa . It is based on the difference of DNA in X and Y chromosomes.

Table 2. Principles of sperm sexing by different methods

S.No.	Method	Basis	Reference
1.	Quinacrine Mustard Staining	Differential Fourescent staining by F body of X and Y chromosome	Barlow and Voss 1970 [3]
2.	Raman Microspectroscopy	Different DNA content between X and Y	De Luca et al. [4]
3.	Centrifugal Counter Current Distribution based on density characteristics	Difference in Density between X and Y	Meistrick(1982)
4.	Albumin Gradient Method	Difference between Albumin Content	Ericsson et al. [5]
5.	Sperm sorting based on volumetric Difference	Difference in sperm head volume	Van Munster et. al.[6]
6.	Swimming pattern under laminar flow	Difference in Swimming pattern of X and Y in any flowing Media	Sarkar et al. [7]
7.	Free flow electrophoresis	Difference in charge on surface of X and Y spermatozoa	Kaneko et al. [8]
8.	Percoll Density Gradient	Difference in Density	Iwasaki [9]
9.	Counter Current Galvanic seperation	Type of Surface charge on X and Y	Bhattacharya, [10]
10.	Immunological Sexing of X and Y	H-Y surface antigent present on the surface of X and absent on Y	Belcher et al. [11]
11.	PCR	DNA difference in X and Y chromosome	Parati et al. [12]
12.	Flow Cytometric	DNA %	Pinkel et. al. [13]

Source- Manzoor et al. [14]

4.1 Procedure for Flow Cytometry

I. Staining of Sperm with non-toxic DNA binding Dye.

II. Pumping it in a stream in front of UV Laser beam having wavelength 351-364 nm and Bright blue flourescent emitted is detected and analysed.

III. This stream is broken by crystal vibrator into individual droplets by facilitating analysis of individual spermatozoa.

IV. The relative flourescence of X and Y chromosome bearing sperm population is analysed by high speed computer which are then sorted by DNA content by introducing opposite charges on droplets containing X and Y chromosome.

V. They are collected separately after electrostatic deflection

5. STATUS OF SEX SORTED SEMEN IN INDIA

According to Kumar et.al. [21] Pashchim Banga Go-Sampad Bikash Sanstha (PBGSBS), a govt. of West Bengal Initiated sex-sorted semen production .First male calf Shreyas was born using sex sorted semen on 1st Jan 2011.Their current production-40-50 sexed semen straws per day. Conception rate-20.7%in cow and 35.3% in Heifer.ABS India is providing Holstein and Jersey sexed semen.Prime Bovine Jersey is providing SSS of HF, Jersey, Brown Swiss, Gir .Planning Commision, Govt of India has assigned production of SSS to NDRI, KARNAL.

Cost-1000-1500/Straw. Due to many religious and social constrains, keeping male calves is totally uneconomical for the poor farmers as there are decreased numbers of male calves buyers. The Stray Male calves and bulls thus produced and left on streets are a big menace and trouble. It will help to reduce cattle vigilantism also. To produce superior breeding bulls Hitherto not much explored application). The projected additional frozen semen doses required per year (millions) is 48, 9.6 and 52.8 for indigenous, crossbred and buffalo respectively [22] It makes the progeny testing more accurate by producing more number of progenies per bull under progeny testing programme thereby increasing the accuracy of bull testing. Coupling it with modern biotechnological methods i.e. assisted reproductive techniques like multiple ovulation, embryo transfer technology, In vitro fertilization, Gamete intra fallopian transfer and sperm intrafallopian transfer will help to overcome the problem of lower fertility associated with it.

6. MERITS OF SEX SORTED SEMEN

It Produces Sex specific calves(with 90% accuracy). Not only does it tackle the problem of replacement of heifers in dairy farms, but also help in Herd size expansion via increased female calf production. In dairy farming, there is surplus production of male calves. Male dairy calves increase the risk of dystocia compared with heifer calves and as an unwanted by-product of breeding with conventional semen, they have a low economic value [23] Due to many religious and social constrains, keeping male calves is totally uneconomical for the poor farmers as there are decreased numbers of male calves buyers. The Stray Male calves and bulls thus produced and left on streets are a big menace and trouble. It will help to reduce cattle vigilantism also. To produce superior breeding bulls(Hitherto not much explored application)- The projected additional frozen semen doses required per year (millions) is 48, 9.6 and 52.8 for indigenous, crossbred and buffalo respectively [22] It makes the progeny testing more accurate by producing more number of progenies per bull under progeny testing programme thereby increasing the accuracy of bull testing. Coupling it with modern biotechnological methods i.e. assisted reproductive techniques like multiple ovulation, embryo transfer technology, In vitro fertilization, Gamete intra fallopian transfer and sperm intrafallopian transfer will help to overcome the problem of lower fertility associated with it.

6.1 Impact of Applying Sex Sorted Semen on the Selection Proportion of the Sire of Dams Selection Pathway in a Nucleus Program

According to Van Vleck [24] a maximum of 15% increase in genetic improvement is expected in a dairy population when sex sorted semen is widely applied. In a dairy cattle breeding scheme using genomic selection, Pedersen et. al. [25] reported a maximum genetic gain of 6% in total merit index when sex sorted semen was applied in both nucleus and production populations. Pedersen et. al. [26] emphasized that the use of

sex sorted semen has limited value on the genetic improvement compared with MOET. Khalajzadeh et. al. [27] reported an average superiority of 9.2% to 11.5% when applying sex sorted semen. They, however, did not find any considerable change in genetic superiority of active sires. Boustan et. al. [28] through a simulation study, predicted an increase of 25 and 34 to 38 percent, after applying sex sorted semen for traditional and genomic evaluation, respectively.

6.2 Predetermination of Sex with 85-90% Accuracy

We can predetermine the sex of calves with 85-90% accuracy by sexing the sperm [29,30] Similar success has been reported in other species [31-35] Sex Predetermination came about due to advances in computer science, biophysics, Cell biology, and applied reproductive physiology [36]

6.3 Herd Expansion

Bio-economic modeling was used to investigate the effect of using sexed semen on either virgin heifers only or on both virgin heifers and lactating cows on herd expansion [37] lactating cows on herd expansion [38] The production of additional heifer calves with sexed semen, as demonstrated by Beutler et.al. [39] can be applied in non-expanding herds to produce heifers for sale. These studies demonstrates that the use of sexed semen, either fresh or frozen, can facilitate faster more profitable herd expansion in seasonal, pasture based dairy herds [39]. According to Kumaon and Kharche, [40] Prediction of sex also offers an advantage in situation when a large number of embryos are needed to establish a herd/ flock of specified genotype compared to introduction of exotic breed/ species.

6.4 Supply of Replacement Heifer

It is an important issue in dairy industry and this constraints is removed by SSS [41]He further adds that selection intensity is increased by choosing genetically superior dams of replacement which accelerate the ratev of genetic gain in dairy herds [16,27] According to Kumaon and Kharche [40] Prediction of sex also offers an advantage in situation when a large number of embryos are needed to establish a herd/ flock of specified genotype compared to introduction of exotic breed/ species.

6.5 Reduced Cases of Dystocia

According to Seidel, [45] use of female sexed semen reduces the dystocia cost in 1st calving heifers by ~ 20%. Amol et. al.[46]. furthers says that sexed semen lowers the risk of dystocia in case of female calf born to medium size heifers because female calf has lower body weight and size than male calf of similar age. So much so that according to Amol et.al. [46] Dystocia and stillbirth for conventional semen in heifer was 6 and 10.4% respectively and in cows it was 2.5 and 3.6%, respectively. These values for sexed semen showed that dystocia declined by 28% in heifers and 64% in cows. Stillbirth frequency was reduced by sexed semen use for cows [42] Use of sexed semen reduced the losses from dystocia in heifers [43]

Increased Rate of Genetic Gain- Sexed semen has been hypothesized as a tool to increased or accelerate the rate of genetic gain in dairy herd. [44,40,16,27]

7. DEMERITS OF SEX SORTED SEMEN

According to Shekereh and Vries 2018), sex sorted semen has a negative impact on the sire of dam (SD) pathway due to increase in selection proportion. Consequently the selection intensity is decreased which further means reduced genetic gain. It has been found that the conception rate is 10-20% lower in sorted sexed-semen compared to conventional semen .High cost of installment of machine and 1000-1500 Rs./Straw. Commercial inavailability of Sex Sorting Technology and lack of skilled and Trained Personnel.There is a need to standardize the lower dosage of spermatozoa and site of deposition for AI with good conception rate under Indian Condition. Diminishing Number of Y-Chromosomes in the population of the concerned breed could eventually lead to degeneration and extinction of Y chromosomes with large unexpected results.Negative impact on effective population size leading to important and long term survival and hence conservation Issue.

8. EFFECT OF SEX SORTED SEMEN ON POPULATION DYNAMICS VIA IMPACT ON EFFECTIVE POPULATION SIZE

Effective population size is given mathematically [47] Ne=1/4Nm +1/4Nf (Approx) So more the N_m: N_f =1:1 ratio deviates from 1:1, more effective the population size Ne closer to Nc (Census Population) and more the chances of its

survival. Falconer and Macay [47] further adds that if te population were maintained with an indefinitely large number of females but only one male in each generation, Effective number would be only four. So reducing the male population would eventually mean that we are decreasing Ne, howsoever the N_c might be thus increasing the chances of threat to survivability to the population.

Infact so much is the relevance of effective population size that according to Wedekind, [48] genetic problems are only indirectly linked to the census size (Nc). Indirectly they are directly dependent on the genetically effective population size(Ne) that is defined as the size of an ideal model population that looses genetic variability at the same rate as the observed population. He further adds that Ne is significantly smaller than Nc because of variance in individual reproductive success, deviations from 1:1 operational sex ratio, and other reasons, risks of extinction are therefore increased if the population sex ratio deviates from 1:1.

According to Wedekind, [49] managing population sex ratios by manipulating ecological or social factors that affect sex-specific growth and survival or that affect life history and hence family sex ratio. The concept is actually so important and relevant that they could aim to support small and endangered populations, either by preventing distorted sex ratio or by inducing small female biased sex ratio distortions to increase N_c of the later generations, even if this means to first reduce N_c in the F_1 and possibly F_2 .

According to Lenze, [50] and Wedekind, [49], the immediate negative effect of such an induced genetic bottleneck would have to be compensated by additional population growth as a consequence of manipulation. The effective population size is the number of individuals that an idealized population would need to have in order for some specified quantity of interest to be the same in idealized population as in the real population.

(http://en.wikipedia.org//org//wiki/Effective_population_size). The concept of effective population size was introduced in the field of population genetics in 1931 by American Geneticist Sewall Wright [51,52]

9. EFFECT OF SEX SORTED SEMEN ON GENETIC ARCHITECTURE OF POPULATION CONCERNED BY DEGENERATION OF Y CHROMOSOME

According to Graves [53] in XY system, mammalian sex chromosome is Highly dimorphic. The large gene rich X and small heterochromatic Y are almost completely differentiated but pair over a small homologous region at one tip (the pseudoautosomal region). He further adds that Y represents a broken down X means accepting that it has lost all but 45 of the about 1000 genes with which it began. In chicken comparison of W and Z tells the same story: of all the genes on chicken Z, only a few remains on W. [54] In mammals, Y seems to be subject to far more mutation, deletion and insertion than the rest of genome. This bias is calculated as a factor of 4.8 in humans [55], accounts for occupation that most De novo dominant genetic diseases arise on father's chromosomes[56] .So then we select against already degrading Y chromosomes in cattle with XY system of sex determination, we should be ready for much faster degradation infact disappearance of Y-Chromosome. As Graves et al. [53] puts it that calculation of rate of loss of genes from Y predicts that sooner or later Y will run out of gene altogether and disappear.

Table 3. Status of dairy animals in India

S.No.	STATES	2016-17 (000 Numbers UNDER AI)	2017-18 (UNDER AI)	Adult Female bovines (000 nos. 2012)	Milk production (000 tonnes, 2017-18)	SOURCE
1	JHARKHAND	714	728	3020	2016	www.nddb.coop
2	UTTAR PRADESH	11360	12659	24501	29052	www.nddb.coop
3	TOTAL	70062	73070	133271	176347	www.nddb.coop

According to H.J.Muller [57] the sex chromosome pair evolve from a pair of autosomes. Mammalian sex chromosome are highly dimorphic. The large gene rich X and the small heterochromatic Y are almost completely differentiated but pair over a small homologous region at one (the pseudoautosomal region. Human X (165 Mb) bears about 1000 genes with a variety of general and specialized functions [58]

Human Y Chromosome is much smaller (60 Mb) and contains a few genes. According to Skaletsky et.al. [59] the euchromatic region receives a total of 178 transcribed units , but many are pseudogenes or amplified copies . The Y encodes only 45 unique protein. In mammals accepting the theory that the Y represents a Broken down X means accepting that it has lost all but 45 of the ~ 1000 genes with which it began .

According to Graves, there is universal acceptance of the hypothesis that Y chromosome degrade but widespread and vigorous debate about the rate at which this occurs and the prognosis for Human Y. Different Models for degeneration lead to different estimates of times at which the human Y will completely run out of genes, estimates of extinction time for human Y ranges from 125000 years to infinity.

10. EFFECT OF SEX SORTED SEMEN ON GENETIC ARCHITECTURE, GENE FREQUENCY OF THE TRAIT/S CONCERNED VIA ARFICIAL SELECTION

According to Falconer and Macay [47], for selection against a recessive gene, $q_1 = (q\text{-}sq^2)/1\text{-}sq^2$;
For s=1 (intensity of selection against the genotype=1), and for all other forces i.e. mutation and migration being assumed to be constant,

Number of generations needed to change q_t to q_0 is-

$q_t = q_0 / (1+tq_0)$

For,

$q_t = (1/n)^* q_0$ i.e. for the gene frequency of the genotype selected against to be reduced to $1/n^{th}$ time the original, $t = (n-1) / q_0$ e.g., if the initial gene frequency of a particular gene is 0.01(say) and the artificial selection is operating against the same genotype (it being assumed to be recessive in nature) with intensity of selection 's', so the time t required in years required to reduce the gene frequency to $1/10^{th}$ of the original can be as follows given by,

$t= (n-1)/q_0 = 10-1/0.01=900$ years and so on.

So the effect of artificial selection, can be shown in the above equation via the reduction in gene frequency. This equation and example also shows the effect of Sex Sorted Semen on the gene frequency of recessive genes present on Y chromosomes.

11. MILK PRODUCTION IN JHARKHAND WITH RELEVANCE OF SSS

According to a report "The Avenue Mail", Jharkhand's own English Daily, Published on 29th Nov, 2018, Niti Aayog CEO Amitabh Kant lauded Jharkhand for registering 19% growth in agriculture sector in last four years. Amul India Managing director R.S. Sodhi said that Jharkhand could generate Rs. 8000/- crores to Rs. 10000/-Crores by boosting daily production of milk by 25 lakh litres. He further added that Jharkhand is a deficit state in milk production. The state produces 52 lakh litres of milk but it requires in addition 75 lakh litres of milk to meet the national average. Milk production of Jharkhand is affected due to non-descript cattle in Jharkhand whose production is very low. Lesser adaptability and extent of A.I. in Jharkhand due to geographical and cultural in uniformity reduces the ambit and scope of Government run schemes . According to Filho et.al. [60] the use of sex sorted sperm has been applied worldwide combined with artificial insemination (A.I.) upon estrus in heifers. He further adds that use of sex-sorted semen during timed A.I. (TAI) programs and for insemination of superstimulated donors for in vivo embryo programs. Pregnancy per AI (P/AI) of cyclic heifers inseminated in estrus with sex sorted semen has been reported to be approximately (75-80%) of the P/AI of heifers inseminated with non-sorted semen.

According to Kouamo and Kharche [40] prediction of sex also offers an advantage in situation where a large number of embryos are needed to establish a herd or flock of specified genotype compared to introduction of of an exotic breed/ species. Predetermination of the sex of offspring could have a significant impact

on livestock breeding and production particularly in selection programme when products e.g. milk comes from one sex only [61,62,63]

12. CONCLUSION

Keeping in view the need to increase milk production to feed the ever increasing population, Sex Sorted Semen Technology is very important like any other biotechnological tools or Assisted Reproductive Technology. In states like Jharkhand, SSS along with IVF and or A.I. will help to boost the dairy sector and help to provide impetus to the milk production. But saying the SSS is without any long or short term side effect on the genetic architecture of the population, would be too early a prediction. Research should be carried out to finally confirm about the effect of SSS on population dynamics and Y-Chromosome degeneration. Large scale SSS scheme coupled with A.I. is needed in many states including Jharkhand but a proper policy must be devised and solutions sought from concerned scientific fraternity to mitigate or nullify the side effect or disadvantages of SSS.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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