Indian Journal of Animal Sciences **88** (3): 265–275, March 2018/Review Article

Evolution of Frieswal cattle: A crossbred dairy animal of India

SUSHIL KUMAR[1], RANI ALEX[2], G K GAUR[3], S S MUKHERJEE[4], D K MANDAL[5], UMESH SINGH[6], S TYAGI[7], ARUN KUMAR[8], A K DAS[9], RAJIB DEB[10], MAHESH KUMAR[11], A S SIROHI[12], NAIMI CHAND[13], RAJENDRA PRASAD[14], V BHASIN[15], B PRAKASH[16] and S KASHYAP[17]

ICAR-Central Institute for Research on Cattle, Meerut Cantt.,Uttar Pradesh 250 001 India

Received: 8 September 2017; Accepted: 18 October 2017

ABSTRACT

Frieswal cattle is one of the crossbred strains having 5/8 Holstein Friesian and 3/8 Sahiwal inheritance, developed by ICAR-Central Institute for Research on Cattle, Meerut, in collaboration with Ministry of Defence. The project was started in 1987 for the evolution of a new crossbred national milch breed: Frieswal – (Holstein×Sahiwal), yielding 4,000 kg of milk with 4% butter fat in a mature lactation of 300 days. It is the only project in the country where such a huge population of animals (more than 20,000) is available with accurate production and pedigree records of each and every animal over generations, with well-established progeny testing network in 37 military farms to cover all the agro-climatic zones of the country. In this review, an attempt has been made to analyze the genesis, present status and future prospects of Frieswal cattle in India.

Key words: Crossbreeding, Frieswal, Holstein, Military farms, Sahiwal

During the last five decades, interest in crossbreeding has increased in several countries due to its well-established benefits (Sorensen *et al.* 2008) due to heterosis, where the crossbred animals are more robust and more efficient in terms of economics in comparison to the parental breeds (Pedersen and Christensen 1989). The systematic crossbreeding programs are running in several countries, among them Denmark, United States and New Zealand are in forefront (Kargo *et al.* 2012).

In order to meet the milk requirement of growing population of India, crossbreeding of local cattle with exotic dairy breeds was adopted as an alternative, since selection and upgrading of large number of non-descript cattle could not bring expected increase at production level in the past decades.The crossbreeding experiments attained momentum in India with the formation of Taylor breed by crossing Zebu with the Jersey breed as early as in 1856 and the efforts are still on to develop crossbred strains by crossing *Bos indicus* breeds with highly developed temperate breeds. The experimental crossbreeding trials were initiated mostly using various exotic dairy breeds at institutional farms. The experiments were focused on various research hypotheses such as which breeds were to be employed; the level of exotic inheritance to which the local non-descript Zebu animals were to be upgraded and the selection policies for crossbreds to be adopted. As a result of these crossbreeding experiments, several dairy cattle strains/breeds have been evolved in various parts of the country, which include Taylor, Jersind, Karan Swiss, Karan Fries and Sunandini. Except Sunandini, these crossbred strains have existed in small numbers in organized farms. Further, it was concluded that crossbreds with exotic inheritance around 50% were suitable in different climatic zones of the country and proved to have 2–3 times more milk production than the indigenous cattle breeds. Since Holstein crossbreds outperformed other crossbreds, they were continued in the country. By late 1970s, the National Commission for Agriculture (NCA) had issued general guidelines to maintain the level of exotic inheritance at approximately 50% in cross-breeding programme. Extensive reviews on crossbreeding experiments in cattle were published by many authors such as Amble and Jain (1966), Katpatal (1979), Bhat (1988), Arora *et al.* (1993) and Taneja *et al.* (2002). The results of various crossbreeding

Present address: [12,13]Senior Scientist (Ajaysirohi35 @gmail.com, n_chand75 @rediffmail.com), Animal Genetics and Breeding Section. [16]Director (birhamp@gmail.com). [3]Principal Scientist (gyanendrakg@gmail.com), ICAR-Indian Veterinary Research Institute, Izatnagar. [4]Principal Scientist (Sabayasachi.Mukherjee@icar.gov.in), ICAR-National Research Centre on Mithun, Nagaland. [5]Principal Scientist (dkmandal1998@gmail.com), ICAR-National Dairy Research Institute, ERS, Kalyani. [8]Principal Scientist (karunt04 @yahoo.com), ICAR- Central Sheep and Wool Research Institute, Avikanagar. [15]Principal Scientist (vineetbhasin.icar@nic.in), Indian Council of Agricultural Research, New Delhi. [17]Deputy Director General (kashyapsudhir.16@gmail.com), Frieswal Project, IHQ, Ministry of Defence, New Delhi. [1,6,7,9,11,14]Principal Scientist (chaturvedisk@gmail.com, usinghas@gmail.com, shrikanttyagi07@gmail.com, achintya137@yahoo.com, mkpdcattle@gmail.com, rp2005pdc@yahoo.co.in), [2,10]Scientist (ranialex01vet@gmail.com, drrajibdeb@gmail.com).

trials were astounding and crossbreds made a significant contribution in making India the world leader in milk production. The crossbreds constitute only 16.6% of the total cattle population but contribute 25.3 million tonnes (53%) of cow milk. In comparison, the indigenous cattle (83.4% population) contributed 22.5 million tonnes (47% of cow milk) (19th Livestock Census 2012, 2014).

Frieswal cattle is one of the crossbred strains having 5/8 Holstein Friesian and 3/8 Sahiwal blood, developed by ICAR-Central Institute for Research on Cattle, Meerut, in collaboration with the Ministry of Defence, GoI. In this review an attempt has been made to analyze the genesis, present status and future prospects of Frieswal cattle in India.

Frieswal-Its development

Zebu taurus *crossbreeding results from AICRP on Cattle:* During the third five year plan a crossbreeding program "Studying behaviour patterns of zebu crossbreds" was initiated on 1 April 1968 at IVRI, Izatnagar and at Hisar centre of PAU, Ludhiana (presently CCSHAU, Hisar), with the base population of 820 Hariana cows which were crossed with Holstein (to bring in genes for fast growth and early maturity), Brown Swiss (to bring in the genes for hardiness) and Jersey (for small size, efficient feed conversion and high fat%). The project was aimed to determine simultaneously the relative performance of various inter crosses and choose an appropriate level of exotic inheritance (1/2 Vs 3/4) with a view to assess their relative suitability for evolving a dairy breed of cattle for arid zone of India. Later this project was merged with All India Coordinated Research Project (AICRP) on Cattle and four more centres were included, one each in Mysore, Maharashtra, Madhya Pradesh and Andhra Pradesh. The coordinating unit of the project was established at IVRI, Izatnagar. Further, an international crossbreeding project supported by the UNDPPL 480 and ICAR at Harringhatta also became part of the All India Coordinated Research Project on Cattle.

As per the government policy, crossbreeding was advocated for non-descript cattle to produce half-bred with exotic donor, which emphasized on equal inheritance from both parents, one for contributing endurance and the other for higher productivity. Further these half-breds were suggested to breed among themselves, *inter se*, in subsequent generations to create large inter mating populations of half-bred. In order to maintain the genetic progress of this population, selection and use of genetically evaluated half bred sires were suggested. Even though the policy did not support the crossbreeding of indigenous cattle breeds of India, the impressive increase in milk production in the crossbreds had resulted in the expansion of crossbreeding programme throughout India, which even spread to breeding tracts of indigenous cattle. In the beginning the exotic breeds used in crossbreeding programme included Jersey, Brown Swiss, Red Dane and Holstein Friesian, but later the choice narrowed to Jersey and Holstein. Results from crossbreeding experiments have been conclusive that Holstein Friesian nicks well with all the Indian breeds, especially with Sahiwal irrespective of the environment. The experiments with other exotic breeds like Guernesy, Ayrshire, Shorthorn, Red Dane, Brown Swiss and Jersey did not produce encouraging results on overall performance basis.

ICAR and military farms

By 1980's, due to the sustained crossbreeding, about 8 million crossbred cattle were born using various temperate exotic breeds. In order to stabilize this National milk herd, around 50% temperate inheritance; proven crossbred bulls of high genetic merit were required. To produce the required number of crossbred bulls it was necessary to fix the genetic value of the resultant new strain. In order to meet these twin objectives, a collaborative national project between ICAR and Military Farms was launched on 23 May 1985 utilizing the crossbred population with 3/8-7/8 Friesian inheritance at Military Farm Meerut (Bhat 1988) by Late General A.S. Vaidya, the then Chief of the Army Staff.

The military farms besides being the largest single source of crossbred cattle in India they served as a source of data for the study of dairy animal improvement through the induction of superior exotic germplasm.The project Directorate on Cattle was established by the Indian Council of Agricultural Research (ICAR) as a nodal institution to monitor, co-ordinate and support all research and development projects for cattle improvement on 3 November 1987 at Military Farms School & Research Centre, Meerut, by upgrading the status of All-India Coordinated Research Project (AICRP) on Cattle. The directorate was conceived to take advantage of the achievements made in the AICRP and other crossbreeding experiments at organized farms especially at military farms and under field conditions for evolution of a national milch breed from a reasonably large crossbred base (Sahiwal × Holstein-Friesian). The directorate has been upgraded to Central Institute for Research on Cattle.

Breeding policy of military farms and implementation of Frieswal project

The initial breeding policy followed at the military farms was crossbreeding, mainly to maintain 3/8 to 5/8 Friesian inheritances. In 1980, alternative breeding policy was formulated and implemented, involving forward crossing with exotic bulls and backcrossing with Sahiwal bulls (Fig. 1).

According to the HF level of the crossbred animals, different genetic groups were developed which formed the base population for developing the Frieswal breed with a HF inheritance of 62.50±5.00%. In the beginning, the crossbred stock with 50% or more exotic inheritance (higher cross) was mated with available half-bred Friesian × Sahiwal bull semen. The lower crosses (cows with less than 50% exotic inheritance) were mated with imported Friesian semen to raise the exotic inheritance of their progeny to

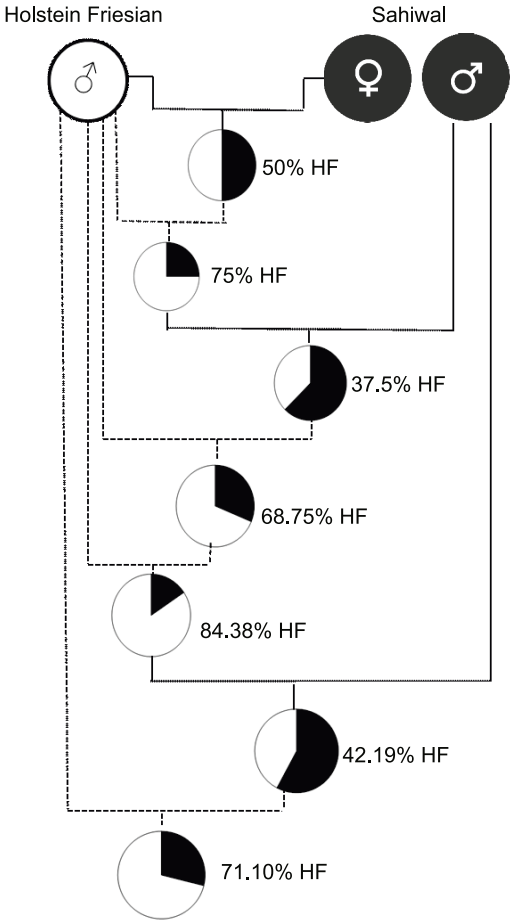

Fig. 1. Breeding policy of military farms in 1980s.

over 50%. All animals in successive generations were bred with 5/8 bulls raised from 3/8 dams, yielding over 3,000 kg milk in a lactation period of 300 days, and mated with imported proven Holstein Friesian semen. Testing and ranking of 5/8 HF bulls is based on their daughters' performance. Intense selection based on the standards set out is being carried out to stabilize the breed. The project started in Military Farm, Meerut with 100 heads of crossbred cattle where breeding commenced on 4 September 1984. Military farms at Jalandhar, Bareilly, Dehradun, Ambala and Lucknow further joined and all other military farms joined the project at a later stage. Initially the project targeted to yield >3,000 litre of milk in 300 days with a peak yield of 15 litre in first lactation, and which in due course of project was elevated to 4,000 kg.

The project was implemented in three phases. In the first phase about 1000 cows in the Military Farm, Meerut were included. This continued till such time the first lactation yield became available. After studying the performance of progenies at Meerut, second phase was initiated. In the second phase about 4,000 cows from 5 more military farms (Lucknow, Dehradun, Jalandhar, Ambala and Bareilly) were included. After studying the first lactation performance of progenies at these farms and of second and third lactations at Meerut, the project entered into the third phase where an extensive genetic evaluation programme involving all the military farms. All cows were included in the programme, but in later stages, the standard of production was suggested to include only cows yielding over 4,000 kg in a lactation of 300 days with 4% butter fat (Anonymous 1991). All the cows having 5/8 Holstein Friesian inheritance were designated as Frieswal cows and these animals were mated with Frieswal bulls for progeny testing of large number of bulls in each cycle (Anonymous 1994).

Frieswal project envisaged evolving a National Milch Breed Frieswal, a Holstein-Sahiwal cross, yielding 4,000 kg of milk with 4% butter fat in a mature lactation of 300 days. The evolution of Frieswal breed of cattle has been progressing utilizing the crossbred herds available at 37 military farms (MFs) located in various agro-climatic regions of the country. Under broader objective Frieswal project is being run with the objectives to study the genetic and phenotypic variance in milk production of Holstein-Sahiwal crossbreds and associated characters related to growth, production, reproduction and survival, and covariances among all these characters with a view to develop suitable selection criteria for improving milk production and to undertake progeny testing of a large number of bulls, their selection on the basis of the genetic merit and utilizing them in military farms and other crossbreeding programmes.

In the ICAR-Central Institute for Research on Cattle, various state of the art laboratories have been established such as semen freezing laboratory, animal nutrition laboratory, animal physiology laboratory and animal breeding and molecular genetics laboratory where research is being carried out to support the project so that the envisaged objectives are fulfilled. Central Institute for Research on Cattle is working in collaboration with Directorate on Frieswal Project which is partner agency for rearing the Frieswal cows and maintaining the bull rearing unit (BRU) at Meerut where male calves born out of elite cows mated with ranked Frieswal bulls are received and reared up to the age of maturity for semen collection and pass through rigorous selection processes. Semen is collected and stored only after proper evaluation of semen quality parameters. Besides this, there are 37 military farms which supply the pedigree and performance data of Frieswal cows located in various agro-climatic regions. However, formal Data Recording Units (DRUs) are located at Meerut, Lucknow, Jhansi and Bareilly in Uttar Pradesh; Ambala in Haryana; Pathankot and Jalandhar in Punjab; Pimpri in Maharashtra; Secunderabad in Andhra Pradesh; Guwahati in Asom; Bengdubi in West Bengal, Jammu in Jammu and Kashmir and Jabalpur in Madhya Pradesh.

Technical programme of the project

The crossbred females with 5/8 (62.5±5) HF inheritances named Frieswal are bred with the semen of their own genetic group. Crossbred females having more than 50% exotic inheritance are named higher crosses and those with less than 50% exotic inheritance named lower crosses. Breeding strategy was devised so that most of the higher and lower

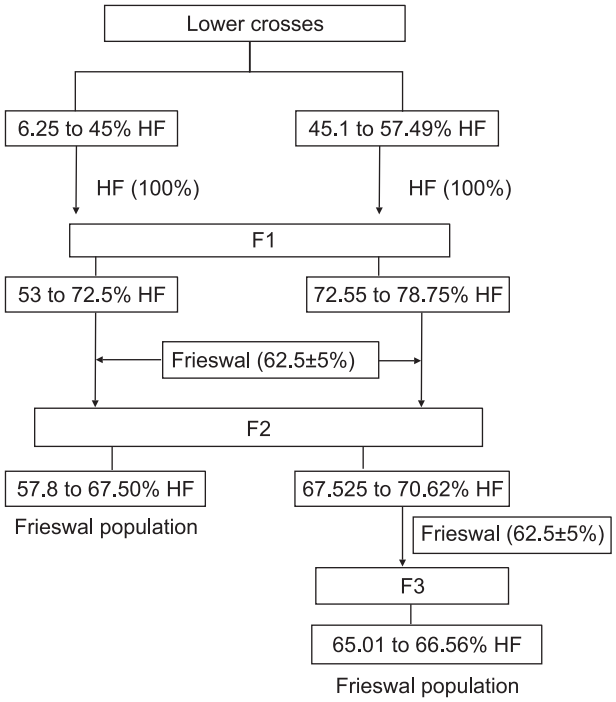

Fig. 2. Illustration of upgradation of lower crosses to Frieswal status

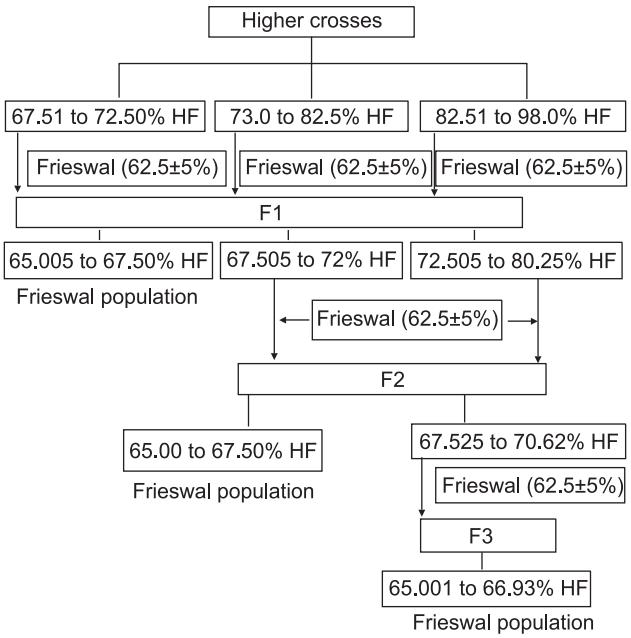

Fig. 3. Illustration of down-gradation of higher crosses to Frieswal status

crosses may be converted to Frieswal animals. Back crossing of higher crosses with Sahiwal semen was stopped and forward crossing of lower crosses with single step HF semen followed by Frieswal semen in successive progenies was advocated (Tyagi *et al.* 2013).

Breeding of cows having 6.25 to 57.49% HF inheritance were given one forward crossing with frozen semen of proven HF bulls with sire index of above 9,000 kg and followed by Frieswal semen usage in all succeeding progenies. Resultant progenies will be converted into Frieswal in second or third generation depending upon the exotic inheritance of the parent generation as shown in Fig. 2. Frieswal cows (62.50±5.0) were mated with Frieswal sires only. Elite Frieswal cows were mated with ranked Frieswal bulls for the production of male calves. Downgradation of higher Friesian crossbred cows was done using Frieswal semen only. Production of Frieswal animals from higher crosses depends on the level of exotic inheritance of the parents and it may take a maximum of three successive mating with Frieswal semen if the exotic inheritance is over 82.5%. An illustration to produce Frieswal animals from higher crosses is shown in Fig. 3.

Males of 3/8 elite cows bred with proven HF semen and 5/8 elite (Frieswal) cows bred with pedigreed/ranked Frieswal semen were screened and put under the test mating. Male progeny born out of elite cows were reared at the respective farms up to 3 months of age for the preliminary selection based on their growth rate and conformation traits. After 3 months, the selected calves were shifted to Bull Rearing Unit (BRU), Meerut for further rearing. Approximately, 15,000 semen straws were frozen depending upon their libido, semen quality, freezability and storage characters from each bull under test. Out of 15,000 straws, 1,000 were kept for gene bank, 4,000 were used for current breeding and 10,000 were stored for future use. Semen of the bulls after evaluation was used extensively to accrue the benefit of proven bulls in genetic improvement.

Achievements of Frieswal project

In India, cattle are raised mainly under small holder farming system with an average of 2 to 3 cows and well organized farms with large number of cattle are very scanty. Lack of proper recording of the animals, especially a pedigreed population in the field further decline the chances of establishing a proper breed improvement programme in the country, as without precise pedigree and production data no selection programme can succeed. The Frieswal Project is the only project in the country where such a huge population of animals (more than 20,000) is available with accurate production and pedigree data for each and every animal over generations. The mixed genotypes of Holstein × Sahiwal crossbreds available in the military farms were converted into Frieswal with 62.5±5% HF inheritance by breeding interventions over several generations. The Frieswal project included all 37 military farms to cover all the agro-climatic zones of the country (Fig. 4). Good performance of Frieswal cows at different locations indicates the adaptation of this germplasm in various agro climatic conditions of the country. Hence this valuable germplasm can be utilized in genetic improvement programme of all parts of the country.

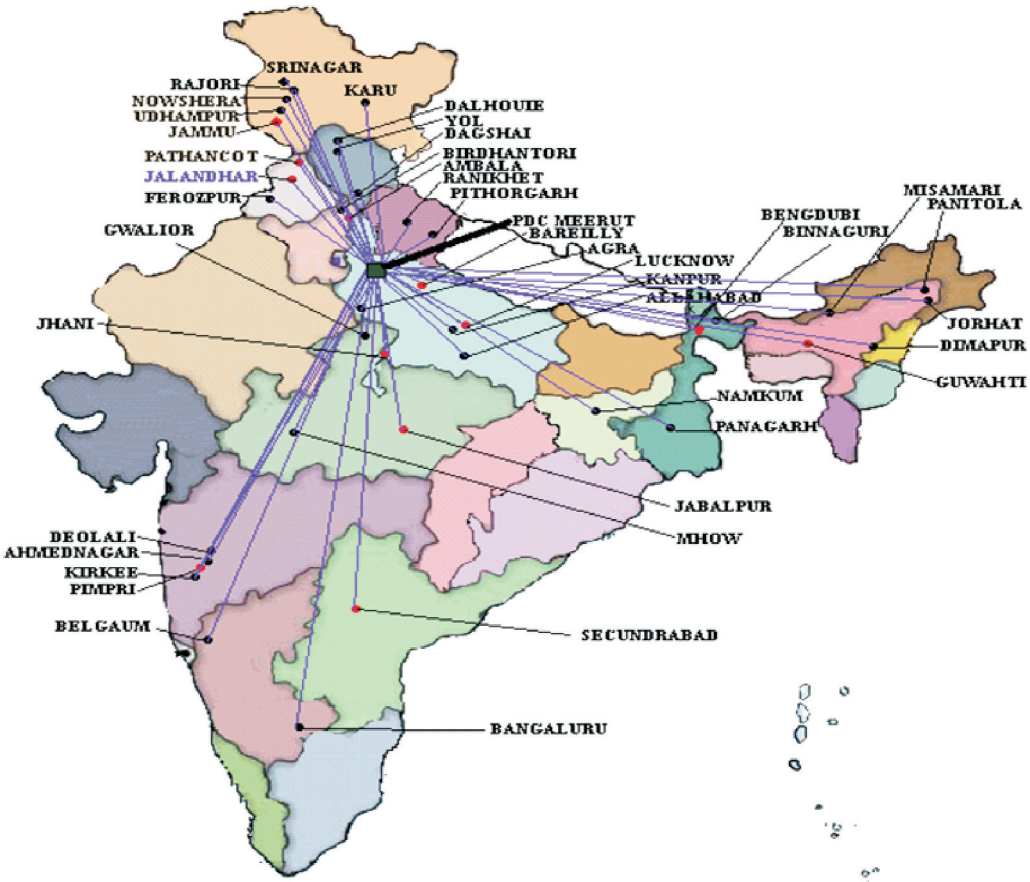

Fig. 4. Location of military farms in various agro climatic zones of India

Population status

The herd strength of Frieswal females increased from 2,305 in 1989 to 20,675 in 2016–17 (Fig. 5). The total population (31 March 2017) of Frieswal females at 36 military farms located in various agro-climatic regions of the country was 20,675 including 11,466 adult cows, 7,153 young stocks and 2,056 calves. The number of Frieswal females was the highest at MF Ambala (2,355) followed by Pimpri (1,699) and Jalandhar (1,424).

Elite cows are identified at the military farms based on the 300-day milk production and peak yield. In the first lactation the animals with a minimum 300 day and peak yield of 4,000 kg and 20 kg, were selected as elite cows where as in second lactation the criteria has been raised to 4,500 kg and 22.5 kg. In the third and subsequent lactations the minimum 300 day and peak milk yield was 5,000 and 25 kg, respectively.The number of elite cows increased to 1,314 in 2016 compared to only 685 in 2005. At present, 11.46% of the adult cows produced more than 4,000, 4,500, 5,000 kg or more milk during first, second or subsequent lactation, respectively (Anonymous 2017). MF Meerut had the highest number of elite cows (131) followed by Ambala (117) and Pimpri (106).

Since inception a total of 1,457 male calves, born out by mating of elite cows with proven bulls and based on breed characteristics and physical conformity at 29 military farms in different years were received at BRU Meerut for selection of young male calves for future breeding. Receipt of male calves as future breeding bulls at BRU increased from a

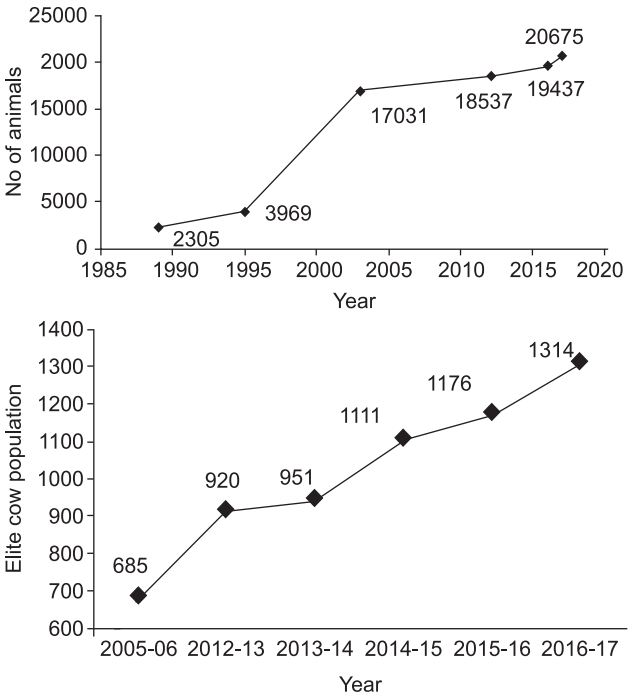

Fig. 5. Trend in herd strength and elite cow population over the years.

mere 260 in 1997–02 to 600 in 2011–16. During the last five years the target of receipt of 100 male calves per year has been successfully achieved, even exceeded.

Phenotypic characterization of Frieswal cattle

The data pertaining to 809 Frieswal animals of different categories was used to describe the physical and morphometric characteristics in Frieswal cattle (Gaur *et al.* 2006). The main body colour of Frieswal animals was black and white in different proportions varying from 10 to 90%. Animals with entire black or tan or tan colour mixed with white have also been observed. Skin colour in majority of animals was black followed by black and brown (Fig. 6). The prominent colour of tail switch was white. Most of the animals had black muzzle (90% cows and 96% heifers). Hoof colour was black in 57% cows and 59% heifers. Adult Frieswal cows were medium (39%) to large in size (56%) with medium dewlap (87%) and small naval flap (93%). Most of the animals were docile (83%). Udder was symmetrical in most of the cows (90%) and heifers (100%).

Frieswal calves averaged 26.51±0.15 kg at birth, 66.69±2.40 kg at 3 months and 117.95±1.70 kg at 6 months of age in females. The average body weight of the heifers was 257±1.68 kg at 18 month and 323±1.63 kg at 24 month of age. Weight at first calving averaged 412.12±2.83 kg. Body height, body length and heart girth averaged 122.37±0.51, 122.23±0.78 and 161.31±0.91 cm in heifers; 129.40±0.32, 122.04±0.41 and 184.36±0.52cm in adult cows and 147.71±0.57 143.25±0.67 and 207.82±0.57 cm in bulls.

Body coat colour and other morphometric characteristics of Frieswal bulls were analyzed using the baseline information of 256 bulls (Mandal *et al.* 2015). Predominant coat colour of Frieswal bull is black patched with white, sometimes brown colour in combination with either black or white. The main muzzle colour of Frieswal bull was black. Another characteristic feature of Frieswal bulls was presence or absence of white spot on forehead. Nearly 40% of the bulls had prominent poll covered with long hair. The other morphometric characters like dewlap, hump characteristics, colour of eyelid colouration on fetlock and hoof and tail switch colour were also detailed (Mandal *et al.* 2015). Morphometric measurements of different body parts and their conformation characteristics were described in different age groups of male animals (Mandal *et al.* 2015). At the age of 3 months, the mean body weight of Frieswal bulls was 103.03±7.43 kg. Body weight continued to increase up to 48 months of age and reached to average 631.73±7.43 kg, thereafter the increment showed fluctuating trends. The bulls attained body weight of 700 kg at 75 months of age, and there after started declining, which is an indication of senility with age advancement.

Fig. 6. Frieswal cow and bull.

Multiplication and dissemination of Frieswal germplasm

A total of 979,975 frozen semen doses of high genetic merit HF bulls were procured from USA, France, Canada and Hessarghatta, Salon and Rishikesh (India) and distributed to different military farms for breeding of the lower crosses. Since inception of the project, a total of 4,356,890 frozen semen doses (31 March 2017) of Frieswal semen have been produced at semen freezing laboratory, out of which 1,388,740 doses have been distributed to military farms. A total of 543,603 doses were distributed to various stakeholders including various State Universities like LUVAS, Hisar and GBPAUA&T, Pantnagar. In addition to these, the Frieswal young or adult bulls were sold to Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, and Uttarakhand state livestock development boards.

Production and reproduction characteristics

The overall least squares means of 300 days milk yield (MY300), total milk yield (TMY), peak yield (PY) and lactation length (LL) were 3312.25±15.94, 3321.74±14.29 kg, 15.11±0.06 kg and 322.02±0.78 days, respectively (Anonymous 2017). The averages of MY300, TMY and PY have increased significantly and the values were 2682, 2785 and 12.8 kg, respectively in 1990–91 (Anonymous 1991). The mature lactation milk yield of Frieswal cows on mature equivalent basis was 3628 kg based on the lactation records of 48,050 lactations spread over more than 25 years (Kumar *et al.* 2017). This was achieved without any culling on production levels in the Frieswal herd. About 35% of total Frieswal cows produced more than 4,000 kg of milk per lactation of 300 days of which 9% produced even more than 5,000 kg per lactation. Analysis of records of 957 male calves received during 2001–2015 revealed that 1.1% of the calves were produced by elite cows yielding 7,000 kg or more milk/300 days lactation; 7.5% calves by cows yielding between 6,000–7,000 kg and 42.8% calves by cows yielding between 5,000–6,000 kg. The survival rate of Frieswal cattle was 76.1% in first lactation. The probability of cows being lost from the herd due to their death and culling was 0.239 at first lactation, 0.2168 in second lactation and 0.2028 in third lactation and decreased as the parity order increased and was lowest (0.09%) in 12th lactation (Kumar *et al.* 2013a).

Milk fat, protein, lactose and solid not fat percentages were 4.11, 3.04, 4.56 and 8.44 of Frieswal cattle. The average fat, protein, lactose and SNF were 4.09, 3.02, 4.54 and 8.42% in morning and 4.13, 3.05, 4.58 and 8.49% in evening milk samples (Anonymous 2012).

The age at first calving also declined to 965.36 days (31.76 months) in 2016, which was 1,005 days (33.06 months) in 2003, thus increasing the total productive life and decreasing the unproductive period. More than 44% of the heifers calved before attaining 2.5 years of age. The

least squares means of service period (SP), dry period (DP) and calving interval (CI) were 164.59, 119.76 and 440.30 days, respectively. The averages of service period and inter calving period in Frieswal cattle were 180.1 and 448.1 days in 1990–91, indicating an improvement over the period. The conception rate of Frieswal cattle was 34.54±1.95 in farms and around 45% in field conditions under Field Progeny Testing (FPT) programme. It was noticed that Frieswal cattle had significantly lower conception rate during rainy season as compared to summer and winter seasons (Mandal *et al.* 2004a). The average milk production of Frieswal crossbred cows is higher than any other crossbred cows produced in the country. This is even more remarkable considering the fact that the milk production data is based on more than 48,000 lactations.

The overall average semen volume (ml), sperm concentration (million/ml), initial motility (%) and post thaw motility (%) were 4.31±0.02, 965.87±5.68, 55.34±0.24 and 45.22±0.30, respectively (Anonymous 2016). Semen production performance of Frieswal bulls revealed that 93.01% of bulls ejaculated semen in artificial vagina and only 45.09% produced freezable semen (Tyagi *et al.* 2000). The seasonal variation in the semen quality parameters was not observed in Frieswal bulls, except semen volume (Mathur *et al.* 2002).

Induction and evaluation of bulls

A total of 260 bulls have been inducted in the programme for progeny testing in the Frieswal project and field progeny testing programme of the ICAR-Central Institute for Research on Cattle. The bulls inducted in different sets were evaluated and rankings were made at fixed intervals (Anonymous 1992, Gaur 2003, Mukherjee *et al.* 2007, Anonymous 2014, Anonymous 2016). The comparative performance evaluation of Frieswal bulls in farms and field conditions were also carried out (Gaur *et al.* 2008 and Kumar *et al.* 2015a). A total of 105 bulls were evaluated based on the performance of daughter's first lactation records. Top 10 bulls had their breeding values between 2,905.24 and 2,993.81 kg. Their superiority over the herd average (2,818 kg) ranged from 87.25 to 175.81 kg (3.10 to 6.24%) (Anonymous 2014).The number of daughters per bull evaluated in the latest set exceeded 50. A full contingent of 30 Frieswal bulls was successfully inducted in 2015 in the progeny testing programme.

Nutritional, management and reproductive interventions

Various research activities have focused on the nutritional, management and reproductive interventions for optimizing productivity of Frieswal cattle. Nutritional studies for optimizing the feeding efficiency of Frieswal bull calves revealed higher growth on the diet formulated based on rumen undegradable protein (RUP) requirements (Anonymous 2013). Oral feeding of combined preparation of estrogen and progesterone along with Agrimin forte @ 40 g/day for 10 days emerged as most economic and convenient method to overcome the problem of anoestrus in Frieswal heifers (Mathur *et al.* 2011, Anonymous 2013). Spermiogram of Frieswal bulls in relation to season and age, seminal attributes and inheritance of seminal quality and sperm abnormality in Frieswal bulls have been established (Mathur *et al.* 2002, Mandal *et al.* 2010a, b, 2012a, b). Age related changes in body size and gonadal development in growing Frieswal bulls were also evaluated (Mandal *et al.* 2004b). Effect of different planes of nutrition and mineral supplementation on nutrient utilization and semen quality of Frieswal bulls (Girdhar *et al.* 2008) and the effect of type of prepuce on semen production performance was also evaluated (Mandal *et al.* 2008). The impact of scrotal skin fold thickness on reproductive traits were evaluated and young Frieswal bulls with less than 4.5 mm scrotal skin fold thickness had superior seminal attributes as compared to bulls having thicker skin fold and this criterion may be preferred when selecting bulls for semen collection.

The problem of high rejection/wastage of crossbred bulls due to poor quality semen production were evaluated under two NAIP projects. Research work under NAIP for understanding the genetic basis of inferior sperm quality and fertility of crossbred bulls was taken up with NDRI, Karnal and IVRI, Bareilly as consortium partners. Under the project, construction of PCR based SSH libraries were constructed. The expression profile of PRM1, PRM2, PRM3, TPN1, TPN2, PKM2 and CATSPER1 genes was studied (Ganguly *et al.* 2013a, Kumar *et al.* 2014b, c). Further, the molecular characterization of SNPs of candidate GnRHR, AQP7, GH, CD9, Clock and Clusterin genes in crossbred Frieswal bulls was also done (Kumar *et al.* 2014b, 2015b). Also, inheritance of semen quality parameters of Frieswal and Vrindavani were estimated (Mandal *et al.* 2012a). The expression of PRM1 gene was significantly higher in good quality bulls than poor bulls while PRM3 was non-significant in good and poor quality Frieswal bulls (Ganguly *et al.* 2013a, Kumar *et al.* 2014c). A significant association of Aquaporin7 (AQ7) gene was found among good and motility impaired Frieswal bulls (Kumar *et al.* 2014a), whereas no significant association was found with growth hormone gene (Kumar *et al.* 2014b). In another project, possibilities to utilize these poor quality semen producer bulls as surrogate bulls for transplantation of sperm producing stem cells from good quality semen producer bulls were investigated. Spermatogonial cells harvested by biopsy of testis from live animal were cultured *in vitro* and the proliferation was estimated by MTT assay. Fetal bovine serum in media was successfully replaced with economically cheap and easily available serum from adult bulls. Effect of growth factors like GDNF, LIF and SCF on growth of SSCs in *in vitro* culture on sertoli cell feeder layer was studied (Chauhan 2014). The spermatogonial cells were enriched using MACS technology and also characterized for the presence of CD9, Oct 4 and DBA (Kumar *et al.* 2013b, Verma *et al.* 2013a, b, 2014). Spermatogonial cells were successfully cultured *in vitro* on sertoli cells to four weeks. Also, three successful passages

of colonies of spermatogonial cells were carried out (Chauhan 2014)

Identification of genetic markers in Frieswal cattle

Various studies were carried out to identify the candidate gene markers and their association with milk production and quality traits (leptin (Singh *et al.* 2013), DGAT1 (Ganguly*et al.* 2013c), prolactin, beta lactoglobulin (Singh *et al.* 2015), kappa casein (Deb *et al.* 2014c) and beta casein (Ganguly *et al.* 2013b)), mastitis susceptibility (calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1) (Deb *et al.* 2014b), breast cancer 1, early onset gene (BRAC1) (Deb *et al.* 2014d), toll like receptor 4 (TLR4) (Deb *et al.* 2013c)), FMD susceptibility (integrin beta 6 (ITGB6) receptor gene (Singh *et al.* 2014)), semen quality traits (protamine 1 (PRM1), protamine 2 (PRM2) (Ganguly *et al.* 2013a), protamin 3 (PRM3) (Kumar *et al.* 2014c), aquaporin-7 (Kumar *et al.* 2014a), CLOCK (Circadian Locomotor Output Cycles Kaput), CLU (clusterin) (Kumar *et al.* 2015b), CatSper1(Kumar *et al.* 2015e), cluster-of-differentiation antigen-9 (CD9) (Kumar *et al.* 2015d), gonadotropin-releasing hormone receptor (GnRHR) (Kumar *et al.* 2015c), Y chromosome microsatellite loci (Deb *et al.* 2013b)) and cellular thermo tolerance (HSP 70 (Deb *et al.* 2013a), HSP 90 (Deb *et al.* 2014a) and ATPase beta family genes (Deb *et al.* 2015, Kumar *et al.* 2016)).

Impact of Frieswal project in farmers' fields

The crossbred cattle in different agro-climatic region of the country are being improved through utilization of high quality germplasm of genetically superior breeding bulls under the Field Progeny Testing programme of the institute. A total 261 bulls has so far been introduced in 14 different sets and total 355,353 inseminations have been done in which 37,308 female progenies were born out of which 10,234 have reached age at first calving with over all conception rate of 43.5%. Presently the programme is implemented in collaboration with Kerala Veterinary and Animal Sciences University, Thrissur, Kerala (KVASU); Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab (GADVASU); BAIF Development Research Foundation, Uruli-Kanchan, Pune; and G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (GBPUA&T).

Inseminations (354,619) were performed in the 4 field centres (100,284 in BAIF; 107,294 in KVASU; 133,452 in GADVASU; and 13,589 in GBPUA&T, Pantnagar), of which 284,848 inseminations were followed for pregnancy confirmation and 124,100 pregnancies were confirmed since inception of the project, with average conception rate as 43.56%. A total of 39,893 female progeny were born at the 4 centres. Through the intervention of Field Progeny Testing programme, the average first lactation 305 days milk yield of the Frieswal progenies in the adopted villages of FPT project has increased by 40.6% at GADVASU, 39.0% at KVASU, 11% at BAIF and 19% in Pantnagar unit (Fig. 7).

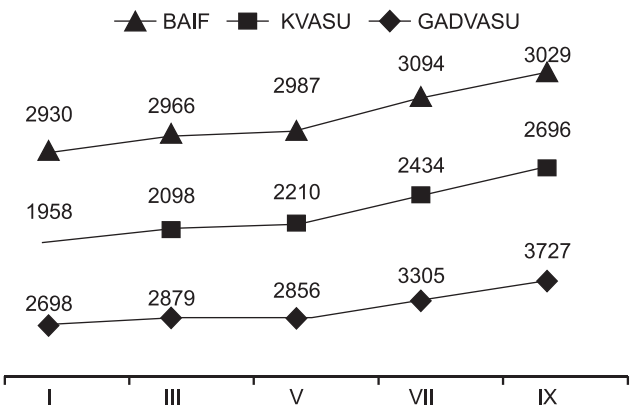

Fig. 7. Improvement in milk yield over the sets in three field centres.

Subsequently average age at first calving of the Frieswal progenies has been reduced by 30% at GADVASU, 16.5% at KVASU, 12.3% at BAIF and 28% in Pantnagar unit. The average lactation yield of progenies was significantly higher than their contemporaries in the respective locations.

The project was started in 1987 for the evolution of a new crossbred national milch animal-Frieswal (Holstein×Sahiwal), yielding 4,000 kg of milk with 4% butter fat in a mature lactation of 300 days. By establishing collaboration between military farms of Ministry of Defence and ICAR-CIRC, livestock resources and infrastructure from the military farms and technical expertise and finances from ICAR, have been brought to a common platform, which helped to utilize Friesian-Sahiwal crossbred as a base for the evolution of a new crossbred national milch animal-Frieswal through *inter se* mating, selection and progeny testing. The utilization of large herds of military farms in various agro-climatic zones of India augmented the production of Frieswal bulls for semen collection and progeny testing by nominated mating of elite cows and proven bulls in the project.

The prime emphasis of the project will continue to be on the evaluation of Frieswal bulls to supply the male germplasm to cater the breeding requirement of the country for increasing the milk production. This will be accomplished by developing the phenotypic databases on production, reproduction and functional traits of cattle both at farm and field levels and the integration of phenomics with the genomics for selection of superior bulls. For these, the project should be continued for a long term, with modifications in the technical programme to incorporate latest technologies developed for more accurate evaluation of breeding males, data generation and analysis and use of various assisted reproductive technologies.

ACKNOWLEDGEMENT

The authors are thankful to Directors, ICAR-Central Institute for Research on Cattle, Meerut, India for providing necessary facilities for conducting the study. We also acknowledge Director, Military Farms and Frieswal Project,

as well as Officer In-charges and staff of various Military Farms for providing necessary information and data. Financial support from ICAR in the form of AICRP on Cattle and National Agricultural Innovation Project is duly acknowledged.

REFERENCES

19th Livestock Census. 2012. *All India Report*. Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Government of India, Krishi Bhawan, New Delhi, India.

Amble V N and Jain J P. 1966. Review of crossbreeding work done in cattle in India. *Seminar on Animal Breeding, Haringhata/Calcutta,* 7–9 December 1966, ICAR, New Delhi. pp 16–63.

Anonymous. 1991. *Annual Report 1990–91.* Project Directorate on Cattle, Meerut Cantt., Uttar Pradesh.

Anonymous. 1992. *Annual Report 1991–92.* Project Directorate on Cattle, Meerut Cantt., Uttar Pradesh.

Anonymous. 1994. *Annual Report 1993–94.* Project Directorate on Cattle, Meerut Cantt., Uttar Pradesh.

Anonymous. 2012. *Annual Report 2011–12.* Project Directorate on Cattle, Meerut Cantt., Uttar Pradesh.

Anonymous. 2013. *Annual Report 2012–13.* Project Directorate on Cattle, Meerut Cantt., Uttar Pradesh.

Anonymous. 2014. *Annual Report 2013–14.* Central Institute for Research on Cattle, Meerut Cantt., Uttar Pradesh.

Anonymous. 2016. *Annual Report 2015 – 16.* Central Institute for Research on Cattle, Meerut Cantt., Uttar Pradesh.

Anonymous. 2017. *Annual Report 2016 – 17.* Central Institute for Research on Cattle, Meerut Cantt., Uttar Pradesh.

Arora C L, Singhal R A, Singh R N and Singh A. 1993. Evolutionary crossbreeding experiments with dairy cattle in India. *Indian Dairyman* **45(9)**: 406–15.

Bhat P N. 1988. Animal Health and Production in Tropics. *Proceedings of the 75th Indian Science Congress, Pune.*

Chauhan M S. 2014. *Characterisation and differentiation of embryonic and spermatogonic stem cell in Cattle and Buffaloes.* NAIP Funded Research Project # 4137. Animal Biotechnology Centre, NDRI, Karnal.

Deb R, Sajjanar B, Singh U, Kumar S, Brahmane M P, Singh R, Sengar G and Sharma A. 2013a. Promoter variants at AP2 box region of Hsp70.1 affect thermal stress response and milk production traits in Frieswal crossbred cattle. *Gene* **532**(2): 230–35.

Deb R, Kumar S, Singh U, Tyagi S, Mandal D K, Sengar G, Singh R, Kumar and Sharma A. 2013b. Evaluation of three bovine Y specific microsatellite loci as alternative biomarkers for semen quality traits in crossbred bull. *Animal Reproduction Science* **142**(3–4): 121–25.

Deb R, Sajjanar B, Singh U, Alex R, Raja T V, Alyethodi R R, Kumar S, Sengar G, Sharma S, Singh R and Prakash B. 2015. Understanding the mechanisms of ATPase beta family genes for cellular thermotolerance in crossbred bulls. *International Journal of Biometeorology* **59**(12): 1783–89.

Deb R, Sajjanar B, Singh U, Kumar S, Singh R, Sengar G and Sharma A. 2014a. Effect of heat stress on the expression profile of Hsp90 among Sahiwal (*Bos indicus*) and Frieswal (*Bos indicus* × *Bos taurus*) breed of cattle: A comparative study. *Gene* **536**(2): 435–40.

Deb R, Singh U, Kumar S, Kumar A, Sharma A, Mann S and Singh R. 2013c. TIR domain of bovine TLR4 gene in Frieswal crossbred cattle: An early marker for mastitis resistance. *Indian Journal of Animal Sciences* **83**(6): 633–35.

Deb R, Singh U, Kumar S, Mann S, Singh R, Sengar G, Kumar A and Sharma A. 2014b. Genotypic to expression profiling of bovine calcium channel, voltage-dependent, alpha-2/delta subunit 1 gene, and their association with bovine mastitis among Frieswal (HF × Sahiwal) crossbred cattle of Indian origin. *Animal Biotechnology* **25**(2): 128–38.

Deb R, Singh U, Kumar S, Singh R, Sengar G and Sharma A. 2014c. Genetic polymorphism and association of kappa-casein gene with milk production traits among Frieswal (HF × Sahiwal) crossbreed of Indian origin. *Iranian Journal of Veterinary Research* **15**(4): 406–08.

Deb R, Singh U, Kumar S, Singh R, Sengar G and Sharma A. 2014d. Profiling of bovine breast cancer 1, early onset (BRCA1) gene among Frieswal (HF × Sahiwal) cows and their association with mastitis. *National Academy Science Letters* **37**: 579–83.

Ganguly I, Gaur G K, Kumar S, Mandal D K, Kumar M, Singh U, Kumar S and Sharma A. 2013a. Differential expression of Protamine 1 and 2 genes in mature spermatozoa of normal and motility impaired semen producing crossbred Frieswal (HF × Sahiwal) bulls. *Research in Veterinary Science* **94**(2): 256–62.

Ganguly I, Gaur G K, Singh U, Kumar A, Kumar S and Mann S. 2013b. Betacasein (CSN2) polymorphism in Ongole (Indian zebu) and Frieswal (HF × Sahiwal crossbred) cattle. *Indian Journal of Biotechnology* **12**(2): 195–98.

Ganguly I, Kumar S, Gaur G K, Singh U, Kumar A, Kumar S and Mann S. 2013c. DGAT1 polymorphism K232A in Sahiwal (Indian zebu) and Frieswal (Holstein Friesian × Sahiwal crossbred) cattle. *Indian Journal of Animal Research* **47**(4): 360–63.

Gaur G K, Kumar A, Beniwal B K and Praveen. 2008. Genetic evaluation of Frieswal bulls under farm and field conditions. *Indian Journal of Animal Sciences* **78**(2): 218–21.

Gaur G K, Kumar A, Singh U, Rathi S K, Singh K and Biswas B B. 2006. Physical and morphometric characterization of Frieswal cattle. *Indian Journal of Animal Sciences* **76**(12): 1030–32.

Gaur G K. 2003. Estimated breeding values of Frieswal bulls for the performance traits. *Indian Journal of Animal Sciences* **73**(1): 79–82.

Girdhar N, Prasad R, Kumar M and Singh K. 2008. Effect of different planes of nutrition and mineral supplementation on nutrient utilization and semen quality of Frieswal bulls. *Indian Journal of Animal Sciences* **78**(7): 764–68.

Kargo M, Madsen P and Norberg E. 2012. Is crossbreeding only beneficial in herds with low management level? *Journal of Dairy Science* **95**(2): 925–28.

Katpatal B G. 1979. Cattle breeding research and application in India – Present and future. *Dairy Cattle Breeding in the Humid Tropics*, Haryana Agricultural University, Hisar, 12–17 February 1979, pp 31–91.

Kumar A, Singh U, Kumar S and Beniwal B K. 2013a. Analysis of Frieswal cattle for survival pattern, herd structure and expected herd life. *Indian Journal of Animal Sciences* **83**(2): 173–75.

Kumar M, Mandal D K, Tyagi S, Mathur A K, Sharma A and Verma J. 2013b. Enrichment of spermatogonial stem cells by using magnate assisted cell sorting and CD9 cell surface

marker. *Proceedings of National Symposium on Physiological and Nutri-genomic Interventions to Augment Food Security and Animal Welfare*. 19–21 November, 2013. DUVASU, Mathura.

Kumar S, Alex R, Singh U, Kumar A and Das A K. 2015a. Comparative performance evaluation of Frieswal bulls in organized farms and farmers' herds. *Indian Journal of Animal Sciences* **85**(3): 316–19.

Kumar S, Alex R, Singh U, Raja T V, Deb R, Alyethodi R R and Prakash B. 2017. Estimation of factors for standardizing lactation yield to mature equivalent basis and factors affecting 305 day mature equivalent milk yield in Frieswal cattle. *Indian Journal of Animal Sciences* **87**(8): 1038–41.

Kumar S, Deb R, Singh U, Ganguly I, Mandal D K, Singh R, Sharma S, Singh G, Singh R, Kumar M and Sharma A. 2014a. SNPs at exonic region of aquaporin-7 (AQP-7) gene may effect semen quality parameters among crossbred bulls. *Journal of Genetics* **93**: 108–12.

Kumar S, Deb R, Singh U, Ganguly I, Mandal D K, Tyagi S, Kumar M, Sengar G, Sharma S, Singh R and Singh R. 2015b. Bovine circadian locomotor output cycles kaput (CLOCK) and clusterin (CLU) mRNA quantitation in ejaculated crossbred bull spermatozoa. *Reproduction in Domestic Animals* **50**(3): 505–09.

Kumar S, Ganguly I, Deb R, Singh U, Mandal D K, Mann S, Singh R, Kumar M and Sharma A. 2015c. Elucidating the role of bovine GnRHR on semen quality traits among crossbred bull. *Indian Journal of Animal Sciences* **85**(12): 1314–17.

Kumar S, Singh U, De S, Mandal D K, Kumar S and Deb R. 2014b. Genetic basis of inferior sperm quality and fertility of crossbred bulls. NAIP Funded Research #4140. Central Institute for Research on Cattle, Meerut, Uttar Pradesh.

Kumar S, Singh U, Deb R, Alyethodi R R, Rani A, Singh R and Prakash B. 2016. *Candidate gene markers: Status and significance for phenomic selection in Frieswal cattle.* Technical Bulletin. ICAR-Central Institute for Research on Cattle, Meerut. 75p.

Kumar S, Singh U, Deb R, Tyagi S, Mandal D K, Kumar M, Sengar G, Sharma S, Singh R and Singh R. 2015d. A SNP (g.358A > T) at intronic region of CD9 molecule of crossbred bulls may associate with spermatozoal motility. *Meta Gene* **5**: 140–43.

Kumar S, Singh U, Ganguly I, Deb R, Singh R, Mann S, Sengar G, Mandal D K, Kumar M and Sharma A. 2015e. Transcript abundance of CATSPER 1 gene in spermatozoa of good and poor quality semen producing Frieswal crossbred bull. *Biotech Today* **5**(2): 47–50.

Kumar S, Singh U, Ganguly I, Deb R, Singh R, Sandeep M, Sengar G, Mandal D K, Kumar M and Sharma A. 2014c. Protamine 3 expressions in crossbred bull spermatozoa may not be a prognostic marker for differentiating good and poor quality semen. *African Journal of Biotechnology* **13**(20): 1999–2003.

Mandal D K and Tyagi S. 2004a. Factors affecting conception rate in Frieswal and other Holstein Friesian × Sahiwal crosses. *Indian Journal of Animal Sciences* **74**(1): 98–99.

Mandal D K, Kumar M and Tyagi S. 2010a. Effect of age on spermiogram of Holstein Friesian × Sahiwal crossbred bulls. *Animal* **4**: 595–603.

Mandal D K, Kumar M, Tyagi S and Kumar S. 2010b. Sperm morphological abnormalities in astheno-oligospermic Frieswal bulls. *Indian Journal of Animal Reproduction* **31**: 52–55.

Mandal D K, Kumar M, Tyagi S and Prakash B. 2015. *Characterization of Frieswal bulls: Body morphometric traits, semen quality and cytogenetic profile.* ICAR-Central Institute for Research on Cattle. 44p.

Mandal D K, Kumar M, Tyagi S, Ganguly I, Kumar S and Gaur G K. 2012a. Pattern of reproductive wastage and inheritance of semen quality in Frieswal crossbred bulls. *Tamil Nadu Journal of Veterinary and Animal Sciences* **8**(5): 245–49.

Mandal D K, Singh K and Tyagi S. 2004b. Age related changes in body size and gonadal development of growing Frieswal bulls. *Indian Journal of Animal Sciences* **74**(1): 31–34.

Mandal D K, Tyagi S and Kumar M. 2008. Effect of type of prepuce on semen production performance of Frieswal bulls. *Indian Journal of Animal Sciences* **78**(11): 1259–60.

Mandal D K, Tyagi S and Kumar M. 2012b. Spermiogram of low vis-á-vis high sperm output crossbred frieswal bulls. *Indian Journal of Dairy Science* **65**(4): 314–18.

Mathur A K, Prasad R and Sharma S. 2011. Efficacy of oral feeding of combined preparation of progesterone and oestrogen on the induction of oestrus in Frieswal females. *Indian Journal of Animal Sciences* **81**(11): 1113–15.

Mathur A K, Tyagi S, Mukherjee S and Singh S P. 2002. Semen quality attributes in Frieswal bulls. *Indian Journal of Animal Sciences* **72**(1): 59–60.

Mukherjee S, Joshi B K and Gaur G K. 2007. Comparison of sire evaluation methods in Frieswal cattle. *Indian Journal of Animal Sciences* **77**(8): 773–76.

Pedersen J and Christensen L G. 1989. Heterosis for milk production traits by crossing Red Danish, Finish Ayrshire and Holstein Friesian cattle. *Livestock Production Science* **23**: 253–66.

Singh R, Deb R, Singh U, Alex R, Kumar S, Chakraborti S, Sharma S, Sengar G and Singh R. 2014. Development of a tetra-primer ARMS PCR-based assay for detection of a novel single-nucleotide polymorphism in the 5' untranslated region of the bovine ITGB6 receptor gene associated with foot-and-mouth disease susceptibility in cattle. *Archives of Virology* **159**(12): 3385–89.

Singh U, Deb R, Kumar S, Singh R, Sengar G and Sharma A. 2015. Association of prolactin and beta-lactoglobulin genes with milk production traits and somatic cell count among Indian Frieswal (HF × Sahiwal) cows. *Biomarkers and Genomic Medicine* **7**(1): 38–42.

Singh U, Kumar S, Deb R, Mann S and Sharma A. 2013. Genotypic profiling of coding region of leptin gene and their association studies on reproductive and milk production traits in Sahiwal and Frieswal cattle of India. *African Journal of Biotechnology* **12**(42): 6140–46.

Sorensen M K, Norberg E, Pedersen J and Christensen L G. 2008. Crossbreeding in dairy cattle: A Danish perspective. *Journal of Dairy Science* **91**: 4116–28.

Taneja V K, Nagarcenkar R and Bhat P N. 2002. Cattle and Buffalo breeding. *Handbook of Animal Husbandry.* Directorate of Information and Publications of Agriculture, ICAR, New Delhi.

Tyagi S, Mandal D K, Prasad R, Kumar S and Nehra R P S. 2013. *Management Practices for Frieswal Cattle*. Project Directorate on Cattle, Meerut Cantt., Uttar Pradesh.

Tyagi S, Mathur A K and Agarwal S C. 2000. Semen production performance of Frieswal bulls. *Indian Journal of Animal Sciences* **70**(10): 1032–34.

Verma J K, Mathur A K, Tyagi S K, Kumar M, Mandal D K and Sharma A. 2013b. Characterization of spermatogonial stem cells (*in vitro*) through nano-magnetic beads targeting CD-9 surface marker in cattle. *Proceedings of National Seminar on*

Biotechnological Approaches to Challenges in Animal Health and Production. 6–7 March 2014. College of Veterinary Science, Mathura.

Verma J K, Sharma A, Mandal D K, Tyagi S K, Mathur A K and Kumar M. 2014. Isolation and enrichment of spermatogonial stem cells with MACS in cattle calves by autopsy *in vitro*. *Advances in Animal and Veterinary Sciences* **2**(3): 183–87.

Verma J K, Sharma A, Mandal D K, Tyagi S K, Mathur A K and Kumar M. 2013a. Use of Western Blotting for molecular characterization of Spermatogonial Stem Cell (SSCs) using CD-9 and Oct- 4 markers in cattle calves *in vitro*. *Proceedings of National Seminar on Biotechnological Approaches to Challenges in Animal Health and Production*. 6–7 March 2014, College of Veterinary Science, Mathura.