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Editorial

21st Dairy Industry Conference: Perspective 1990*

XXI Dairy Industry Conference, 12-14 September, 1985 was hosted by Dr. V. Kurien, Chairman, N.D.D.B. and Chairman of the Organising Committee, at the National Dairy Development Board, Anand the capital of the dairy world of India. While inaugurating the XXI Dairy Industry Conference and wishing all success in its deliberations Shri Yogendra Makwana, Minister of State for Agriculture, Government of India, said that while our major thrust in dairy development must remain through co-operatives, there is much that is required to be done by our Departments of Animal Husbandry and by our Government owned Research Institutes to render support of farmers to increase their milk production and solve many problems for collecting, processing and marketing of milk. All concerned institutes must direct their research increasingly towards problem oriented research and also quickly transfer the findings from their laboratories to the farmers and dairies.

Shri B.P.S. Puri, President, Indian Dairy Association pointed out the significance that the theme of this year's conference, "Prespective 1990" would emphasize Marketing, Research & Development and Education and Training as the thrust areas for the massive programme which is expected to double the existing turnover of the organised dairy industry in the next five years.

Dr. M.P.G. Kurup, Executive Director (FIT), National Dairy Development Board stated that the demand for milk by 2000 AD will be 80 MMT. In comparison, the availability of green fodder, dry fodder and concentrates would be 575.00, 356.80 and 77.05 MMT respectively by 2000 AD. To achieve the productivity targets set under the proposed strategy, he advocated an integrated approach to milk production. All the available tools of genetic improvement including artificial insemination, embryo transfer technology, progeny testing, biotechnology and genetic engineering, computers and statistical tools to be utilized and integrated to obtain the optimal genetic gain through possible paths of genetic improvement i.e. sire-sire, sire-dam, dam-sire and dam-dam. Use of urea molasses block to increase utilization of available crop residues and reduce pressure on concentrates. Also, the adoptation of by-pass protein technology in feed formulation to reduce the requirement of concentrates still further. Development of common grazing lands and waste lands for fodder production within the society villages to over come the deficiency of green fodder. He concluded that use of advance technologies in animal breeding, animal nutrition and fodder production and integrating them

to our advantages and implementing through co-operative milk marketing system would lead to technological revolution in our villages resulting into more incomes for producers and better quality of life in rural area.

The main features of the strategy for increasing milk production in the country by the Government of India are: cross-breeding of non-descript low producing cattle with exotic dairy breeds, progressive genetic improvement of important buffalo breeds, development of feed and fodder resources, organisation of effective animal health services and enlarging inputs and marketing infrastructure under Operation Flood. Also genetic improvement of important indegenous cattle breeds by selective breeding in their home tract and upgrading in other areas which is important is being done.

Editorial Board

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* Source: Proceedings, XXI Dairy Industry Conference, 1986.

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Immunological Studies On The Origin Of Seminal Plasma Proteins Of The Indian Buffalo

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ABSTRACT

The origin of seminal plasma specific proteins from the vas deferens, head, body and tail epididymis and testes of the Indian buffalo was immunologically investigated in sixty four healthy buffalo bulls using rabbit antibuffalo seminal plasma specific serum and immunodiffusion analysis. At least 10 to 12, 8 to 10 and 5 to 6 seminal plasma specific proteins originate from the vas deferens, epididymis and testes respectively of the buffalo bull. These proteins were of slow, medium and fast diffusion rates. Some of the proteins originated from the vas deferens, head, body and tail epididymis and testes were antigenically similar while other proteins originating from these organs were different. Structural similarities and differences of these protein molecules originating from different parts of the reproductive tract of the buffalo bull could he according to physiological/biochemical requirements of spermatozoa for their maturation, acquisition of the property of forward progressive motility and capacity for fertilization.

* * *

The heterogenecity of seminal plasma proteins and their vital role in the maturation of spermatozoa, their acquisition of the property of forward progressive motility and capacity for fertilization has been established recently (Acott and Hoskins, 1978, 1981, 1983; Acott *et al.*, 1983; Klinefelter and Hamilton, 1984; Hoskins *et al.*, 1979). In view of this, extensive studies on the origin of seminal plasma proteins (Wong *et al.*, 1981; Clavert *et al.*, 1980, 1985; Balerna *et al.*, 1984) their identification and characterization (Brook and Higgins, 1980; Toowicharanout and Chulavatnatol, 1983; Kato *et al.*, 1985) hormonal regulation of their synthesis and secretion by the epididymis and testes (Carreau *et al.*, 1980; Mongkolsirikieat and Chulavatnatol, 1983, 1984; Holms *et al.*, 1984; Galdieri *et al.*, 1984) in men and animals have been reported more recently. The biological and immunological roles of testicular and epididymal proteins and proteins of the accessory sex glands in the sperm physiology of domestic animals have been reviewed (Matousek, 1985). Low survial rate and poor fertilizing capacity of refrigerated/deep frozen spermatozoa of the Indian buffalo (*Bos bubalus bubalis*) as compared to cattle spermatozoa (*Bos indicus*) is an important economic problem of Indian dairy industry (Anand 1979). However, very little information is available on seminal plasma proteins of the Indian buffalo (Dhanda *et al.*, 1982; Kulkarni, 1984, 1985, 1986). Information on the origin of seminal plasma proteins of the buffalo and their specific role in sperm physiology

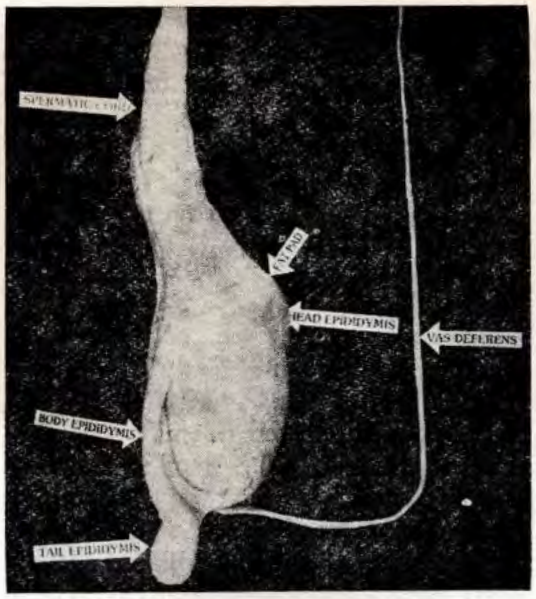

Fig. 1. The testis, epididymis, spermatic cord and vas deferens of the Indian buffalo.

is not available. The present paper reports the origin of seminal plasma specific proteins of the Indian buffalo from the testes, epididymis and vas deferens using specific antiserum against these proteins and immunodiffusion analysis.

Materials and Methods

Collection and processing of reproductive organs of buffalo bulls:

Sixty four pairs of testes alongwith the spermatic cord were collected from apparently healthy Murrah buffalo bulls immediately after slaughter at Deonar Abattoir, Bombay and were transported to the laboratory. The organs were washed under tap water and different parts *viz.*, epididymis, testes and vas deferens were dissected out and processed as stated in brief:

Epididymis:—Epididymis from each testis was exposed after separating the tunica

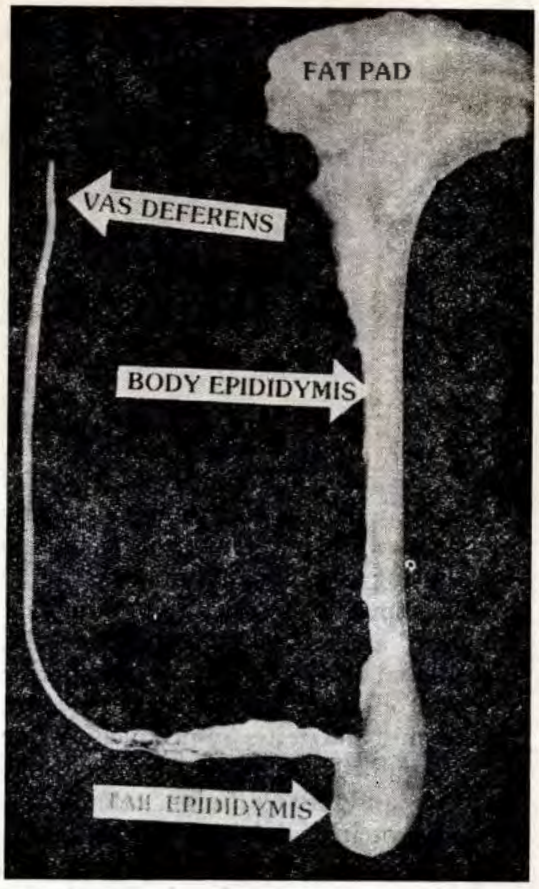

Fig. 2. Fully exposed epididymis and vas deferens of the Indian buffalo.

albuginea from the testis and was divided into three parts *viz.*, the head, body and tail and were cleared of adherent fat and connective tissue. Each part was separated, cut into small pieces with scalpel and transferred into an aliquote of 15 ml of phosphate buffered saline (PBS) pH 7.4 and kept at 4°C overnight. The PBS containing pieces of the head, body and tail epididymis were centrifuged at 5000 rpm for 20 minntes, the supernatent of each part was pooled and was concentrated to about 200 fold by polyethelene glycol 6000 dialysis and was stored at

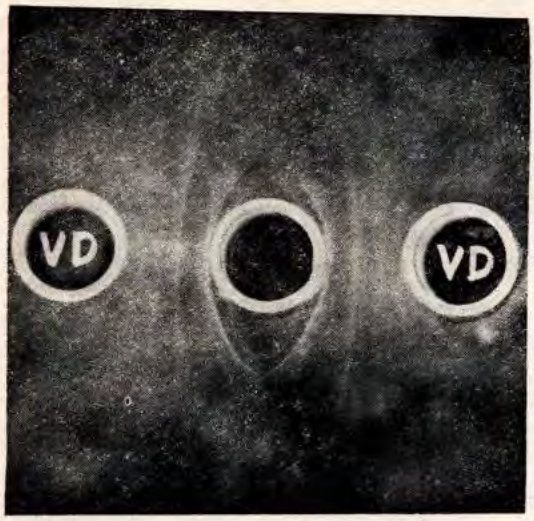

Fig. 3. Ouchterlony plate showing proteins of the vas deferens of the Indian buffalo. Antigen wells (VD) contain washing fluid of the vas deferens. Central well contains absorbed rabbit antibuffalo seminal plasma serum.

Fig. 5. Ouchterlony plate showing proteins of the body epididymis of the Indian buffalo. Antigen wells (BE) contain extract of the body epididymis. Central well contains absorbed rabdit antibuffalo seminal plasma serum.

Fig. 4. Ouchterlony plate showing proteins of the tail epididymis of the Indian buffalo. Antigen wells (TE) contain extract of tail epididymis. Central well contains absorbed rabbit antibuffalo seminal plasma serum.

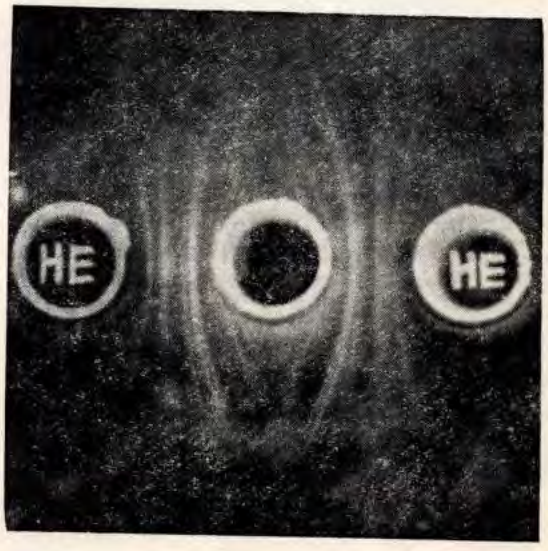

Fig. 6. Ouchterlony plate showing proteins of the head epididymis of the Indian buffalo. Antigen wells (HE) contain extract of the head epididymis. Central well contains absorbed rabbit antibuffalo seminal plasma serum.

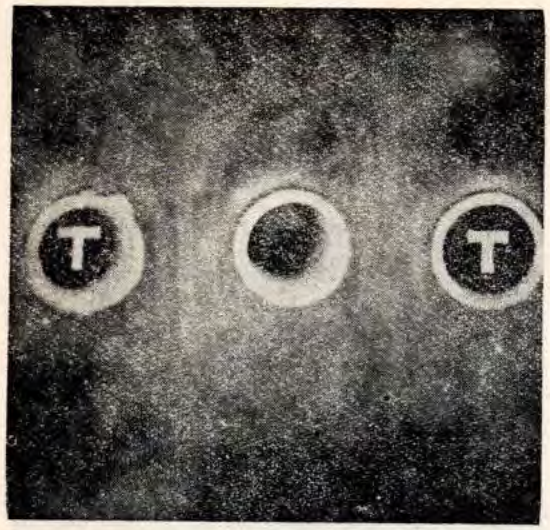

Fig. 7. Ouchterlony plate showing proteins of the testes of the Indian buffalo. Antigen wells (T) contain washing fluid of the testes. Central well contains absorbed rabbit anti-buffalo seminal plasma serum.

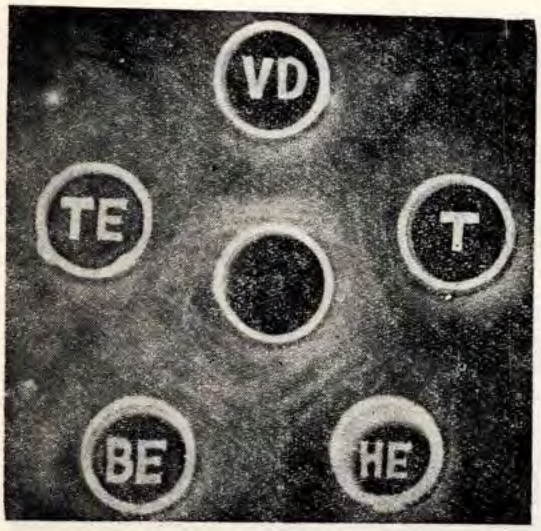

Fig. 8. Ouchterlony plate showing antigenic relationship between seminal plasma specific proteins of the (VD) vas deferens (TE) tail epididymis, (BE) body epididymis, (HE) head epididymis and (T) testes of the Indian buffalo. Central well contains absorbed rabbit antibuffalo seminal plasma serum.

−15°C to −20°C till used for immunological analysis.

Testes:—After separating the epididymis each testis was cut vertically into two halves and was washed with 25 ml of PBS pH 7.4 using a 10 ml all-glass syringe, stored at 4°C overnight, centrifuged, the supernatent was pooled, concentrated and stored as mentioned above till used.

Vas deferens:—About 25 to 50 cm piece of the vas deferens was separated from each spermatic cord and the contents of the lumen of the vas deferens were flushed out by 5 ml of PBS pH 7.4 using a 2 ml all-glass syringe and 20 gauze needle. The vas deferens was cut into 1 cm pieces and transferred in the flushed out PBS and stored at 4°C overnight. The flushed out PBS alongwith the pieces of vas deferens were centrifuged, the supernatent was pooled, concentrated and stored as above till used.

Antiserum:—Adult white rabbits weighing above 2.5 kg were hyperimmunized subcutaneously at multiple sites with pooled buffalo seminal plasma (diluted 1:4 with normal saline) with equal volume of Freunds' complete adjuvant for a period of 3 months. The details of the collection of semen and preparation of seminal plasma have been reported earlier (Kulkarni, 1984). On the 8th day of the last injection rabbits were bled, clear serum was separated by centrifugation and was stored at —15°C to —20°C till used. This antiserum was referred to as rabbit antibuffalo seminal plasma serum.

Preparation of rabbit antibuffalo seminal plasma specific serum:

In order to remove the antibodies against blood serum proteins present in the buffalo seminal plasma (Kulkarni, 1985) rabbit antibuffalo seminal plasma

serum was absorbed with buffalo blood serum. This absorbed antiserum was referred to as rabbit antibuffalo seminal plasma specific serum which will detect only the specific proteins synthesized by the organs of the reproductive tract of the buffalo bull. The details of the preparation of the rabbit antibuffalo seminal plasma specific serum used in the present study have been reported earlier (Kulkarni, 1985).

Immunodiffusion analysis:

Immunodiffusion analysis was done using 0.5% agarose gel in phosphate buffered saline pH 7.4 according to the method of Ouchterlony (1958). In order to obtain intense precipitin lines in immunodiffusograms, the antigen and antibody trenches were refilled 8 times at the intervals of 72 hrs with respective immunoreactants. All immunoplates were developed at 40°C. Washing and staining of immunoplates was done with Amido Black 10-B, using standard method (Crowle, 1973).

Results and Discussion

The dissected testis alongwith the spermatic cord, epididymis and vas deferens, and fully exposed epididymis and vas deferens of the Indian buffalo are presented in Figs 1 and 2, respectively.

Immunodiffusion analysis:—The results of immunodiffusion analysis of the washings/extracts of the vas deferens, tail, body and head epididymis and testes using rabbit antibuffalo seminal plasma specific serum are shown in Figs 3 to 7, respectively. These figs. clearly indicate that the seminal plasma specific proteins of the Indian buffalo partly originate from the vas deferens, epididymis and testes as reported in other species (Matosek, 1985). From these figs., it is also evident that the seminal plasma specific proteins of slow, medium and fast diffusion rates (higher, medium and lower molecular weight) are synthesized by the vas deferens, head, body and tail epididymis and testes of the buffalo. These proteins are transported in the semen at the time of ejaculation.

Proteins of the vas deferens:

Immunodiffusogram of the washings of the lumen of the vas deferens of the buffalo revealed the presence of at least 10 to 12 seminal plasma specific proteins (Fig. 3). Out of which, at least 3 to 4 proteins with slow, 2 with medium and 3 to 4 proteins were with fast diffusion rates. Wenstrom and Hamilton (1984) reported the synthesis and secretion of proteins by the vas deferens of the rat. On the basis of autoradiographic study, these workers have shown at least 6 major protein bands and the fusion of several closely spaced protein bands in the same molecular weight class. Electron microscopic study of the rat epididymis has shown variations in the cytoarchitecture of vas deferens in different regions (Hamilton *et al.*, 1977; Hamilton and Cooper, 1978) which could be the basis of the synthesis and secretion of proteins of different types by cells of the vas deferens. A new glycoprotein "Clusterin" has been reported in the luminal surface of the vas deferens of the rat which aggregates cells (Pierre and Fritz, 1985). The results of the present study have clearly established that the seminal plasma specific proteins of the buffalo partly originate from the vas deferens. Further studies on the identification and characterization of these proteins are essential to understand the sperm physiology of the buffalo.

Epididymal Proteins:

The protein spectra of the extracts of the head, body and tail epididymis of

the buffalo as revealed by immunodiffusion analysis are presented in Figs. 4, 5 and 6 respectively. From these figs., it is evident that the proteins from the three different parts of the epididymis differ in respect of their number and diffusion rates. At least 4 major proteins with fast and 4 to 5 proteins with medium diffusion rates were observed in the extract of tail epididymis. Five to seven proteins have been reported in the fluid of tail epididymis of various species (Amann *et al.*, 1973; Clavert *et al.*, 1985). A high molecular weight glycoprotein "Immobilin" has been indentified in the fluid of tail epididymis of the rat (Usselman and Cone, 1983) which keeps spermatozoa immobilized during their sojorn at the tail epididymis. The difference in the number of proteins in the fluid of tail epididymis of different species could be due to species specificity and partly due to variations in the methods used for protein analysis by different workers. At least 3 to 4 proteins with fast and 1 to 2 each with medium and slow diffusion rates were observed in the extract of body epididymis of the buffalo (Fig. 5).

Immunodiffusion analysis of the extract of the head epididymis of the buffalo revealed the presence of at least 5 to 6 proteins with fast and 1 to 2 proteins each with medium and slow diffusion rates (Fig. 6). Bovine sperm forward motility protein is synthesized by the head epididymis (Acott and Hoskins, 1978) and it's physiological role in the maturation of spermatozoa in the epididymis and the development of forward progressive motility by immobile spermatozoa under conditions of elevated levels of cyclic AMP in the cattle bull has been demonstrated (Brandt *et al.*, 1978; Acott and Hoskins, 1981, 1983; Acott *et al.*, 1983). It is not known whether any structural quantitative differences in the sperm forward motility protein of the buffalo bull exists as compared to cattle bull which may be implicated in the differences of sperm physiology of these two species.

Testicular Proteins:

Immunodiffusogram of the washings of the testes have shown the presence of at least 5 to 6 proteins of slow, medium and fast diffusion rates (Fig. 7). At least 4 seminal plasma specific proteins of testicular origin *viz.*, androgen binding proteins (Ritzen *et al.*, 1973; Carreau *et al.*, 1979; Keeping *et al.*, 1984) transferrin (Steven and Michael, 1984; Holms *et al.*, 1984) clustrin (Pierre and Fritz, 1983) cellular retinol binding protein (Kato *et al.*, 1985) have been identified and characterized. However, their specific role in sperm physiology is yet to be established clearly. The secretion of androgen binding proteins by Sertoli cells is influenced by the contact with germ cells (Galdieri *et al.*, 1984). The seminal plasma specific proteins of testicular origin of the buffalo have not been identified and characterized and need to be studied.

Antigenic relationship between proteins of the vas deferens, epididymis and testes:

The results of immunodiffusion analysis of the proteins of the vas deferens, tail, body and head epididymis and testes of the buffalo showing antigenic relationship between these proteins are shown in Fig. 8. From this fig., it is evident that the proteins of the vas deferens, tail, body and head epididymis and testes show structural similarities in some proteins and differences in other proteins originated from these organs. Distinct structural diffrences have been reported

in the protein profiles synthsized and secreted by the different parts of the rat epididymis (Brooks, 1981). Structural similarities and differences of seminal plasma specific proteins originating from different parts of the epididymis, vas deferens and testes are probably according to the physiological/biochemical requirements of spermatozoa for their maturation, acquisition of the property of forward progressive motility and their capacity for fertilization.

Acknowledgements

The author wishes to thank the officer-in-charge of large animal unit, Deonar Abattoir, Bombay for the supply of reproductive organs of buffalo bulls and Dr. P. D. Sardeshpande, Associate Dean, Bombay Veterinary College, for providing facilities for the present studies.

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IJAR 7: 2: 9-13, 1986

Buffalo Seminal Antibodies: Its Detection And Titration By Gelatin Agglutination And Tube Slide Agglutination Tests*

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ABSTRACT

Female albino rabbits were immunized against buffalo semen, seminal plasma and washed spermatozoa. Elicitation of immune response one week after the subcutaneous injection of the antigen was detected and the antibody titre was assayed by GAT and TSAT. Subsequent build-up of titre following each weekly injection was also assayed by the same tests. The tests were found to be reliable and repeatable for detection and titration of rabbit anti-buffalo seminal-serum.

* * *

A test evolved by Kibrick *et al.* (1952 b) for detection of spermatozoal heteroantibodies and now known as Gelatin Agglutination Test (GAT), has been made use of subsequently by other workers for detecting presence of spermatozoal autoantibodies in the blood serum of sterile men (Rumke and Hellinga, 1959). The test is also seen adopted for detecting seminal antibodies in bovine (Menge, 1967) and in rats (Rumke and Titus, 1970). Similarly, a microtechnique evolved by Franklin and Dukes (1964) for detecting spermatozoal antibodies in the serum of infertile women and now known as Tube Slide Agglutination Test (TSAT), has been successfully adopted by later workers.

The present study was conducted with rabbit anti-buffalo seminal-serum with a view to assess the usefulness of the above tests to detect and quantitate the antibodies against buffalo semen, so that, they can be adopted for detecting and quantitating spermatozoal antibodies in the serum of buffaloes when they occur spontaneously.

Materials and Methods

Buffalo semen for immunization of rabbits, was collected fresh every time by artificial vagina from 7 healthy Murrah bulls and was utilized as immediately as possible after collection. A pretreatment sample of serum was obtained of the blood that was collected from each of four female albino rabbits by cardiac puncture and preserved at —20°C. Once a week for 3 weeks, each rabbit was injected subcutaneously with 0.5 ml of whole semen emulsified in 0.5 ml of Freund's complete adjuvant (FCA). Subsequently at weekly interval two injections of 0.5 ml of whole semen without adjuvant, were given. In another group of four does, two were injected subcutaneously with 0.5 ml

* Part of the thesis submitted by the senior author for the award of Ph.D. Degree of the Tamil Nadu Agricultural University.

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TABLE 1: Titration of rabbit antisera against whole semen, seminal plasma and washed spermatozoa of buffalo by GAT and TSAT.

Order of injection of antigen	Post-injection weekly serum sample	Reciprocals of titre of rabbit serum against buffalo semen		buffalo seminal plasma		bufiffalo sperms	
		GAT	TSAT	GAT	TSAT	GAT	TSAT
First	I	8	16	4	8	8	16
Second	II	64	256	32	128	64	256
Third	III	128	1024	128	1024	128	1024
Fourth	IV	1024	4096	1024	4096	1024	4096
Fifth	V	2048	8192	2048	8192	2048	8192

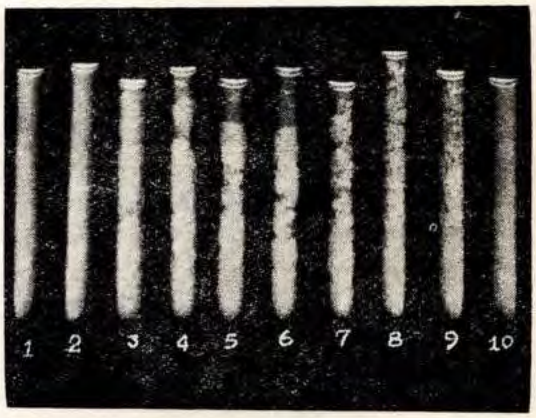
of seminal plasma emulsified with an equal quantity of FCA once a week for 3 weeks and subsequently with 0.5 ml of seminal plasma alone at weekly interval on two occasions; and the other two were injected in the same order and manner with 0.5 ml of spermatozoa washed thrice with normal saline and then emulsified with an equal volume of the adjuvant. Serum samples were collected from each rabbit by drawing 10 ml of blood the day before each weekly injection to study the build-up of titre of agglutinins. A week after the last injection, serum samples were collected from them and were preserved at —20°C. Serum antibody titre in the samples was determined both by GAT and TSAT.

Results

Results of titration of rabbit antiserum against semen, seminal plasma and washed spermatozoa of buffalo by GAT and TSAT are presented in table—1.

In GAT, positive reactions were characterized by opaque clumps of spermatozoa suspended in clear medium (Fig. 1). Intensity and size of clumps had a direct relationshp to the antibody titre. On the contrary, control tubes showed a uniform homogenous appearance. Specific agglutination requires the presence of high percentage of motile spermatozoa when clump formation was quicker and was more distinctly discernible. Gelatin being viscous pre vents disruption of week unions.

Fig. 1 Gelatin agglutination test



1 & 2 control—two fold serial dilutions of rabbit serum 4 & 8.

3 to 10 Experimental—two fold serial dilutions from 4 to 512 of rabbit anti-buffalo seminal serum.

In TSAT, not only intensity but type of agglutination also were observed. Head-to-head agglutination was noticed in all the samples. Highest titre in which agglutination of not less than 70 per cent of the total spermatozoa had occurred, was taken to be positive. In specific agglutination, orderly clumpswere

formed with their free ends directed towards the periphery (Fig. 2) whereas, in non-specific agglutination, disorderly clumps were formed. Though aglutination could occur at ambient temperature it was hastened at 37°C. Size of clumps varied depending on the concentration of antibodies and duration of incubation.

Fig. 2. Specific spermatozoal agglutination

It can be seen from the table 1 that spermatozoal agglutinins against whole-semen were gradually built-up and they were detectable at 1 in 8 to 1 in 2048 with GAT. Whereas, with TSAT they were detectable at 1 in 16 to 1 in 8192 titre from first to fifth week.

Serum titre of agglutinins against seminal plasma obtained at the end of first and second week post-injection was one grade lower than that of whole semen and of washed spermatozoa. Subsequently, build-up of titre was much like that of the other two.

The agglutinins against seminal plasma were detectable at 1 in 4 to 1 in 2048 with GAT, while they were observable at 1 in 8 to 1 in 8192 titre with TSAT from first to fifth week.

Build-up of serum titre in rabbits against washed spermatozoa was similar to that of whole semen with either of GAT and TSAT.

Discussion

Following the discovery by Landsteiner (1899) of the immunogenic property of bull spermatozoa, Metchnikoff (1900) well documented that secretions of various parts of the male reproductive tract contain active immunogens which when injected into heterologous species induce production of antibodies. Rao and Sadri (1960) produced antibodies to buffalo semen by injecting it subcutaneously once weekly for seven weeks into rabbit. In the present expriment the method adopted by De Fazio and Ketchel (1971) for producing rabbit anti-human seminal plasma-sera was followed by emulsifying the antigen in equal quantity of FCA since it influences the type and sequence of antibody production by stimulating all systems of immune response. Subsequent to first three injections, antigen alone was administered to boost up the titre. Injection at multiple sites instead of at single site was adopted as recommended by Menge and Protzman (1967) since it is more effective than the latter.

Mittal *et al.* (1965) reported the bull semen to be strongly antigenic in rabbits. Build-up of serum titre in rabbits in the present investigation indicates the buffalo semen also to be highly immunogenic. One week after injection, there was elicitation of immune response as evidenced by the serum titre of 1 in 16 and thereafter, a steady build-up of titre was recorded following each weekly injection to attain by the end of fifth week, a titre of 1 in 8192.

Agglutination of spermatozoa can be evaluated either by macro or microscopic examination of antigen—antibody suspension. However, macroscopic examination did not distinguish specific from non-specific agglutination. Hence, Mudd (1927) proposed modification recommending centrifugation and gentle shaking of tubes to resuspend the clumps in the medium wherein control in contrast to experimental tube, will remain uniformly homogenous. Kibrick *et al.* (1952a) suggested use of good quality semen besides using narrow tubes (5×65 mm) for effective resuspension of clumps. Even after modification the test was not sufficiently sensitive. They (1952b) further modified it by incorporating gelatin in the sperm suspension to prevent disruption of weak unions. The test so modified became useful for clinical investigation. Rumke and Hellinga (1959) adopted it as a diagnostic aid in the investigation of infertile men. Later it was adopted in bovine (Menge *et al.*, 1962); in rabbits (Menge and Protzman, 1967) and in rats (Rumke and Titus, 1970). Menge (1967) made use of the test for detecting iso-antibodies in the serum of heifers immunized with bull spermatozoa. Now the test is known as GAT and it was found to be equally useful for testing rabbit anti-buffalo seminal-serum also.

Microscopic examination of antigen-antibody suspension for agglutination was described by Henle *et al.* (1938) and termed it as slide agglutination test. Snell (1944) used it in mouse; but, the one proposed by Franklin & Dukes (1964) and now known as TSAT is universally adopted. Clumped cells of not less than 10 percent of the total in the sample is taken as positive. It was adopted by Schwimmer *et al.* (1967) to investigate sera of women for presence of spermatozoal antibodies. The test was utilized in the present investigation and was found to be reliable and repeatable with rabbit anti-buffalo seminal-serum. It was also found to be more sensitive than the GAT.

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Frozen Semen And Fertility In Surti Buffaloes*

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ABSTRACT

An attempt was made to study and know the influence of sire, season/month and nature & duration of oestrus cycle on Conception rates based on 1500 frozen semen inseminations performed in 808 Surti buffaloes over one year period (1984-85) at a village—Bedva. The semen samples collected from 17 Surti buffalo bulls and frozen in tris yolk extender at CSCS, Amul Dairy, Ode (Anand) were utilized for the purpose.

Among the 687 freshly inseminated and 594 overall buffaloes that could be followed, the first service and overall conception rates were 30.88% (212) and 69.69% (414), respectively. A highly significant ($P < 0.01$) difference was observed between months for fresh inseminations' conception rates, being lowest (5.66%) in month of June and highest (44.26%) in month of December. There was a significantly negative correlation between monthly conception rates and monthly average Temperature-Humidity-Index (THI). In general, the conception rate following inseminations during high breeding season (Sept.-Feb.; 38.03%) was 2½ times greater than that in low breeding season (March-Aug.; 14.69%).

The first service conception rates between 17 bulls studied varied highly significantly from 9.09 to 61.11%.

The buffaloes conceiving with I, II, III, IV and V or more repeat inseminations/cycles showed CRs of 30.88, 41.95, 34.15, 34.92 and 56.52%, respectively. The corresponding proportion among the total buffaloes conceived (414) was 51.21, 30.01, 10.16, 5.31 and 3.11%. This indicated that more than 80% buffaloes conceived within 2 inseminations while remaining 20% took more number of services to settle. The overall number of inseminations required per conception averaged 2.71.

Among 532 cycle lengths studied in 335 buffaloes, the proportion of mini (<9 D), mid (9-12 D), short (13-17 D), Normal (18-24 D), long (25-35 D), double (36-42 D) and multiple of normal cycle was 3.71, 3.26, 5.55, 30.55, 25.00, 13.92 and 17.69%, respectively. Of the 184 buffaloes that conceived, the corresponding CRs were 3.26, 4.35, 3.21, 34.24, 26.66, 13.55 and 14.98%. The greatest number of buffaloes conceived with normal cycle lengths.

Of the total 414 duffaloes that conceived during the year, 18 (4.37%) buffaloes resulted in abortion mostly during hot summer months. The sex

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ratio of male to female births observed was 151: 159 (49.97: 50.03) among 310 calvings that could be followed.

* * *

The perusal of scientific literature did not reveal much information on the influence of sires, months/seasons of inseminations and cyclic aberrations on the conception rates of frozen semen in buffaloes especially under field conditions. The literature on the ratio of male and female calf born due to frozen semen inseminations is also scarce. Dutta *et al.* (1980) reported that the months and seasons have effect on conception rates in cattle. Suryaprakasam *et al.* (1985) reported significantly lower conception rates in duffaloes following frozen semen inseminations during summer season as compared to rainy and winter seasons. Due to paucity of informations on these aspects the present study was undertaken under field conditions in Surti breed of buffaloes.

Materials and Methods

The present study was conducted on 808 Surti buffaloes/heifers at one of the College Ambulatory Clinic Centres, Bedva (Anand). A total of 1500 (808 fresh and 692 repeat) frozen semen inseminations were performed by a trained inseminator and by the staff of the Gynaecology Department, Veterinary College, Anand during their Ambulatory Clinic services at the place throughout the year. The period of study covered one full year commencing from 1st April, 1984 to 31st March, 1985. The semen samples from 17 Surti buffalo bulls frozen in French medium straws using standard tris yolk glycerol extender at the Central Semen Collection Station, Amul Dairy, Ode (Anand) were utilized at random for the insemination purpose. The pregnancy diagnosis was carried out after 60-75 days of last insemination by per-rectal examination.

The appearance of clean, glassy, stringy mucus discharge, frequent micturation, withholding of milk and presence of matured graafian follicle on either of the ovary were considered to be the optimum time of insemination at mid cervix. The daily and monthly insemination records along with the name of sire, identity of female etc. and subsequent follow-up till calving/abortion/sale were maintained. The data on climatic environment during the period of study were obtained from Meteorology Department, B.A. College of Agriculture, Anand and Temperature-Humidity-Index (THI) was calculated after Oliver (1973).

Further, to know the occurrence of cyclic aberrations and its effect on conception rates, a total of 532 cycle lengths were analysed in 335 buffaloes that took three or more inseminations to settle. Of the resultant pregnancies

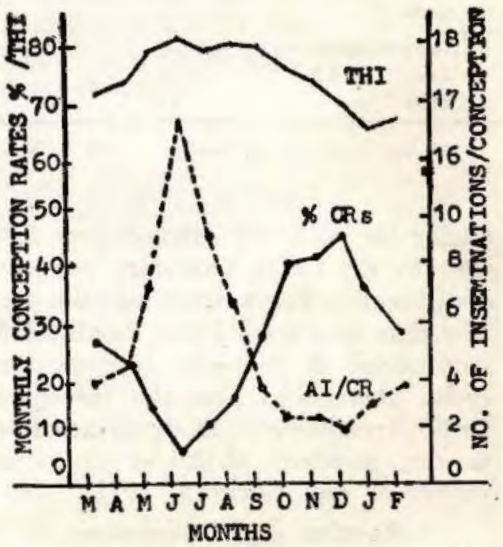

Fig. 1. Monthly average Temperature Humidity Index(THI), first inseminations' conception & no. of inseminations/conception.

TABLE 1: Month-wise frozen semen inseminations and their fertility results in Surti buffaloes as affected by monthly average THI.

Month	Inseminations		Total No. of buffaloes inseminated	No. of buffaloes followed for P.D.		No. found pregnant		Conception rate%		Av. THI	No. of AI/Conception for fresh AI
	Fresh	Total		Fresh	Total	Fresh	Total	Fresh	Total		
March	29	108	100	19	75	5	19	26.32	25.33	72.78	3·80
April	56	57	56	52	52	12	12	23.08	23.08	73.94	4·33
May	17	26	26	15	23	2	3	13.33	13.04	80.43	7.50
June	61	73	71	53	65	3	3	5.66	4.62	82.42	17·67
July	47	71	64	38	50	4	5	10.53	10.00	80.31	9·50
Aug.	44	79	72	34	62	5	8	14·71	12.90	81.20	6.80
	254	414	389	211	327	31	50	14.69	15.29	78.35	6.81
Sept.	67	134	123	52	90	14	21	26.92	23.33	80.77	3.71
Oct.	76	161	151	62	125	25	52	40.32	41.60	77.39	2.48
Nov.	109	195	186	101	170	42	80	41.58	47.06	74.48	2.38
Dec.	140	252	233	122	218	54	90	44.26	41.28	70.02	2.26
Jan.	111	211	200	97	183	34	80	35.05	43.72	66.73	2.85
Feb.	50	133	130	42	121	12	41	28.57	33.88	68.28	3.50
	553	1086	1023	476	907	181	364	38.03	40.13	72.95	2.63
Overall	808	1500	808	687	594	212	414	30.88	69.69	75.99	3.24

The overall number of inseminations per conception = 2.71.

TABLE 2. Analysis of variance showing the effect of months and inseminations on conception rates.

Sources of variance	D.F.	S.S.	M.S.	'F' Value
Inseminations	1	9.7665	9.7665	0.744NS
Months of AI	11	4488.6098	408.0554	31.075**
Error	11	144.4460	13.1312	—

** Significant at 1% level, NS = Non-significant.

during the year, 310 calvings were followed to determine secondary sex ratio of calf born to frozen semen inseminations. The data so obtained and tabulated for month-wise & bull-wise inseminations, cyclic aberrations and the subsequent fertility results were analysed according to the standard statistical procedures (Snedecor and Cochran, 1971).

Results and Discussion

The month-wise fresh & total inseminations, number of buffaloes inseminated, followed & found pregnant and their fertility results have been presented and compared with the monthly average THI in Table-1 & Fig. 1.

The 'F' test analysis (Table-2) revealed highly significant differences in the conception rates following inseminations performed in different months of the year. For the fresh and overall inseminations, the conception rates averaged 30.88 and 69.69%, respectively requiring on an average 3.24 and 2.71 inseminations per

conception. The monthly average THI was significantly and negatively correlated with monthly conception rates for both fresh (–0.678) and overall (–0.693) inseminations. The lowest conception rates (5.66 & 4.62%) were observed in month of June when THI was highest (82.42%). Similarly, highest conception rates were observed in cooler months of November (47.06%) and December (44.26%) when THI was low. In general, the conception rates for inseminations performed in the months of high breeding season (Sept.—Feb.; 26.92 to 44.26%, Av. 38.03%) were 2½ times greater than those obtained in months of low breeding season (March-Aug. 5.66 to 26.62%, Av. 14·69%). These findings closely agreed with the observations of Amir *et al.* (1982) who reported the pregnancy rates to be significantly higher in cows inseminated during winter (47.2 to 56.7%) than in cows inseminated during summer (30.8 to 48.0%) regardless of season of semen collection and storage duration in liquid nitrogen. Suryaprakasam *et al.* (1985) also reported similar trend of CRs in Murrah buffaloes. Ahmed *et al.* (1982) reported the overall requirement of number of inseminations per conception in buffaloes to be 1.77 to 2.75, whereas Jani *et al.* (1984) reported it to be 2.69 under field conditions. Bhavsar *et al.* (1986) found highly significant and negative correlations between monthly conception rates and ambient temperatures in Mehsana and Murrah buffaloes. Bodinga *et al.*(1986) found sharp decline in conception rates among cows when maximum air temperaure exceeded 30°C on the day of AI. They also observed marked seasonal variation in fertility and a negative curvilinear relationship between conception rate and rainfall on the day after AI. The present finding supported the observations made by above authors.

The conception rates obtained under the present study compared well or were higher than those reported by Ganguli (1974) as 43.2%, Takkar *et al.* (1980) as 36.12%, Kottaya and Rao (1978) as 44.8%, Chinnaiya *et al.*(1979) as 46.22%, Vasanth (1979) as 45%, Tuli *et al.* (1981) as 35.22%, Patil *et al.*(1981) as 47.06%, Heuer (1982) as 54.3%, Radhakrishna *et al.*(1984) as 48.61%, Jani *et al.*(1984) as 57.24% and Bhavsar *et al.* (1986) as 44.91%using semen frozen in tris estender. However, Pavithran *et al.*(1972) & Roy (1974) reported 80% and 85.9% CRs, respectively based on non-return rates in buffaloes. Similarly, the first service conception rates reported as 40-50% by Nagarcenkar (1979), 45.8% by Mehar Singh *et al.*(1980), 57.2% by Zhou (1981), 42.6% by Austin *et al.* (1981) and 50.9% by Suryaprakasam *et al.* (1985) were comparatively higher than the present findings (30.88%). Chinnaiya and Ganguli (1980) reported the first service and overall conception rate of frozen buffalo semen extended in tris as 31 and 40%, respectively, which was in agreement with the present finding.

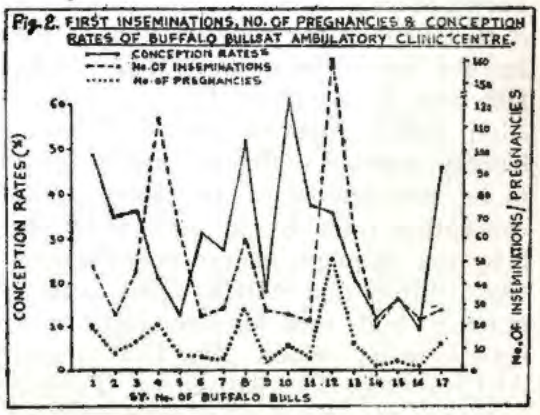

Fig. 2. FIRST INSEMINATIONS, NO. OF PREGNANCIES & CONCEPTION RATES OF BUFFALO BULLS AT AMBULATORY CLINIC CENTRE.

TABLE 3. Sire-wise fresh inseminations and their fertility in buffaloes.

Sr. No.	Name of bulls	No. of fresh AI.	No. of buffaloes followed	No. found pregnant	Fertility rate %	No. of AI/ conception	Month of inseminations
1	Akabar	48	41	20	48.78	2.05	December
2	Banshi	25	23	8	34.78	2.87	January
3	Bhikhu	46	36	13	36.11	2.77	September
4	Bhupat	114	97	21	21.65	4.62	May, June, July
5	Dinu	64	54	7	12.96	7.70	July, August
6	Gopal	24	19	6	31.58	3.16	October
7	Jain	28	18	5	27.77	3.60	March
8	Jalu	60	54	28	51.85	1.93	November
9	Kamal	27	22	4	18.18	5.50	August
10	Kanti	25	18	11	61.11	1.64	January
11	Keshu	22	16	6	37.50	2.66	February
12	Manu	158	142	51	35.84	2.75	Nov., Dec., Jan.
13	Nabi	63	57	12	21.05	4.75	April
14	Rajat	19	17	2	11.79	8.50	February
15	Rakesh	33	25	4	16.00	6.25	September
16	Roshan	24	22	2	9.09	11.00	January
17	Valu	28	26	12	46.15	2.16	December
	Overall	808	687	212	30.88	3.24	—

The first service conception rates of 17 Surti buffalo bulls under the present study varied highly significantly ranging from 9.09 to 61.11 % with the corresponding number of inseminations /conception as 11.00 to 1.64 (Table 3, Fig. 2) These findings are in agreement with those reported by Rao (1976), Kodagali and Rao (1983), Jani *et al.* (1984 & 1986), Suryaprakasam *et al.* (1985) and Bhavsar *et al.* (1986) as regards variations in the fertility rates between buffalo bulls. However, Kottaya and Rao (1978) and Abhi (1982) reported the variation in fertility rates of different buffalo bulls to be non-significant. In general, the conception rates for all bulls were low following summer season inseminations (Av. 19.76 % and AI/CR, 5.06) as compared to bulls used for inseminations in high breeding season (Av. 37.13 % and AI/CR, 2.69). The parusal of Table-3 indicated that the fertility rates of 2 or more bulls used for inseminations in the same month (i.e. September 36.11 %vs 16.00 %; November 51.85 % vs 35.84 %; January 34.78 % vs 61.11 % vs 9.09 % and February 37.50 % vs 11.79 %) also varied greatly proving that the bulls varied in their fertilizing ability. The low conception rates for all bulls in summer indicated that the interaction effect between bulls and females inseminated was responsible for low CRs. It also indicated that buffaloes under field condition showed seasonal breeding behaviour which perhaps lowered CRs of frozen semen inseminations in summer regardless of season of semen production and freezing, as pointed out by Suryaprakasam *et al.* (1985) and Amir *et al.* (1982).

As regards the buffaloes conceiving with I,II,III,IV and V or more repeat inseminations/cycles (refer Table-4), the conception rates were 30.88, 41.95, 34.15-34.92 and 56.52 %, respectively. The

corresponding proportion among the total buffaloes conceived (414) during the year was 51.21, 30.01, 10.16, 5.31 and 3.11%. This indicated that 81.22% buffaloes conceived within 2 inseminations while rest of 18.78% took more number of services to settle. The major population of buffaloes was inseminated and found pregnant during cooler months of September to February, which is a peak breeding season for this breed.

The occurrence of cyclic aberrations and conception rates during these cycles have been presented in Table-5 and in Fig.3. It can be seen that the incidences of normal and long cycles were much higher in buffaloes which subsequently resulted in higher conception rates as compared to other aberrant cycles and their conception results. The occurrence of mini, mid and short cycles indicated problems of delayed or anovulation and development of new crop of follicles on the ovary which may be ovulatory or anovulatory. Some of these cycles were fertile giving low conception rates following inseminaions in such cycles. The higher incidence of double and multiple cycles indicated missed, unobserved or silent heats which were physiologically normal and thus were responsible for more conceptions when subsequently detected and inseminated. The pattern of cyclic aberrations observed under the present study compared well with the cyclic aberrations reported by Derashri (1982), Kavani *et al.* (1984) and Purbey and Singh (1984) in buffaloes. However, these authors have not studied the conception rate during such cycles.

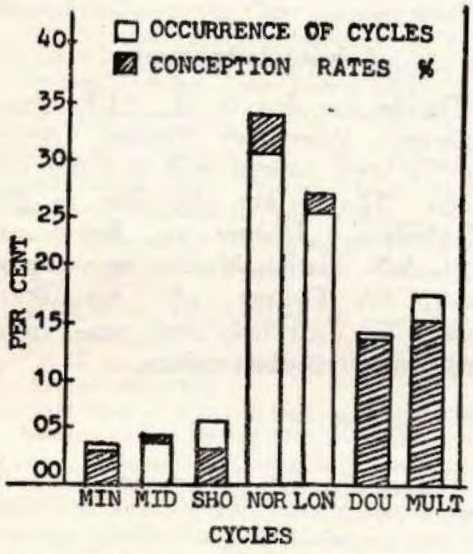

Fig. 3. Incidence of cyclic aborrations and its influence on conception rates in buffaloes.

Based a total 414 buffaloes that conceived during the year, the abortion rate observed was 4.37%(18) in buffaloes. Most abortions were noticed in hot summer months during second trimester of pregnancies. This finding coincided well with the observations of Luktuke and Purbay (1985) who reported 4.44%

TABLE 4. Insemination-wise fertility of frozen semen in buffaloes.

Sr. No.	Particular	Inseminations					
		I	II	III	IV	V & more	Overall
1.	No. of buffaloes inseminated	808	475	173	81	41	808
2.	No. of buffaloes followed	687	298	123	63	23	594
3.	No. found pregnant	212	125	42	22	13	414
4.	Conception rate %	30.88	41.95	34.15	34.92	56.52	69.69
5.	No. of AI/conception	3.24	4.76	8.78	11.45	9.12	2.71
6.	Proportion of the total buffaloes conceived	51.21	30.01	10.16	5.31	3.11	100.00

TABLE 5. Aberrations in oestrus cycle lengths and fertility in 335 buffaloes.

Sr. No.	Nature of cycle length in days	Occurrence		Fertility rate	
		No.	Per cent	No.	Per cent
1.	Mini cycle (< 9 days)	20	3.71	6	3.26
2.	Mid cycle (9-12 days)	23	4.26	8	4.35
3.	Short cycle (13-17 days)	29	5.55	6	3.21
4.	Normal cycle (18-24 days)	162	30.55	63	34.24
5.	Long cycle (25-35 days)	133	25.00	49	26.66
6.	Double cycle (36-42 days)	75	13.92	25	13.55
7.	Multiple of normal cycle	95	17.69	27	14.98
	Overall total	532	100.00	184	100.00

abortions in buffaloes. However, Patel and Kodagali (1985) reported the incidence of abortion in Surti buffaloes as 2.04% and Chatterjee *et al.* (1984) as 9.7% in bovines. In the present study based on 310 calvings, the male to female births recorded were 151 to 159 (49.97:50.03), the difference being non-significant. Shukla and Parekh (1984) observed the overall secondary sex ratio of male to female as 1.06:1 in Gir and its crosses with Holstein and Jersey. Chourasia *et al.* (1985) studied 678 calvings in Murrah buffaloes and reported the sex ratio of male to female as 53.83:46.17 which was not differing significantly from 50:50 ratio. The present findings are thus well in accordance with these observations.

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Effects Of Seasonal Variations On Frozen Semen Production Of Surti Buffalo Bulls

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ABSTRACT

The five years data of twenty Surti buffalo bulls were divided into three seasons on the basis of local climatic conditions. They were Rainy (June to September), Winter (October to January) and Summer (February to May) Seasons. The data were analysed for semen volume, colour and consistency, sperm density, initial activity and sperm motility before and after freezing in a Randomized Block Design.

There was no significant difference observed in semen volume, colour and consistency, sperm density and initial activity due to seasonal and months variations. However, there was an improvement during the winter season. The highly significant difference was observed in sperm motility before and after freezing in winter season. In general, the sperm quality was superior during winter and January. While it was affected during summer and March. Thus the high atmospheric temperature, humidity and wind speed might be affecting the semen quality.

* * *

According to F.A.O. (1978) report, India possesses 47 per cent reverine buffaloes of the world buffalo population, which is one third of the cattle population in India. Efforts are being made to store the superior germ plasm of different breeds of buffaloes, under different agroclimatic conditions. Hence, the present study was envisaged with Surti breed under local climatic conditions.

Materials and Methods

For the study five years (from 1981-85) farm data of twenty Surti bulls (5-10 years old) have been used. The year was divided into three seasons as follows.

A. Rainy season — from June 1st to September 30th

B. Winter season — from October 1st to January 31st

C. Summer season — from February 1st to May 31st.

The climatic factors such as temperature, relative humidity, evaporation, rainfall and wind speed are given in Table-1.

All the bulls were maintained under optimal feeding and management conditions. From each bull, the semen was collected twice a week by using artificial vagina (A.V.) on a male dummy. The collected semen was assessed for its volume, colour, consistency and was observed under binocular microscope of 20X, for its density (1D to 4D) and mass activity (1+to4+). On the basis of its density and mass activity the semen was extended by using tris egg yolk extender along with antibiotics. The motility of the extended semen was observed under microscope. The extended semen was

TABLE 1. Average values of climatic factors in different seasons.

Particulars	Rainy season	Winter season	Summer season
Maximum temperature (°C)	27.76±0.34	29.23±0.22	34.94±0.39
Minimum temperature (°C)	20.09±0.14	15.57±0.48	18.84±0.40
Relative humidity (%)	87.08±0.78	73.33±1.87	65.77±1.75
Evaporation (mm)	3.48±0.25	4.88±0.22	7.38±0.24
Rainfall (mm)	96.48±12.62	22.59±8.77	38.63±12.11
Wind speed (Kmph)	14.22±0.66	6.98±0.19	9.74±0.63

TABLE 2: Average values of semen quantity and quality in different seasons.

Particulars	Rainy season	Winter season	Summer season
Volume (ml)	5.39±0.39	5.53±0.43	5.36±0.43
Density (D)	2.99±0.07	3.09±0.09	3.04±0.08
Mass activity (+)	3.03±0.07	3.12±0.07	2.99±0.06
Motility after dilution and before freezing (%)	76.39±0.33	79.84±0.39**	75.02±0.48
Motility after freezing (%)	44.09±1.21	48.28±1.00**	41.56±1.79

** Significant at 0.01 level

filled in French medium straws (0.5 ml capacity). These straws were frozen by vapour freezing method and stored under liquid nitrogen. After freezing, the motility of frozen semen was observed under microscope at different intervals.

The data so obtained was processed statistically in a Randomized Block Design by Snedecor and Cochran(1967) method.

Results and Discussion

The differences due to seasons in the average values of semen donated by bulls were statistically non-significant (Table-2). There was no significant difference in semen volume between months. The semen donated between the bulls was higly significant. These results were in accordance with the findings of Kodagali (1962) in Khillar bulls, but contradictory to the findings of Kushwaha *et al.* (1955), Patel (1959), Gill *et al.* (1974) and Bhosrekar (1980) in Murrah buffaloes. It could be due to change in climatic factors and the breed.

There was no statistical difference in the colour and consistency due to seasons, months and bulls. The colour and consistency of the semen donated by the bulls ranged from thin milky to thick milky. These observations were in agreement with the observations of Mukherjee and Bhattacharya (1952).

On the perusal of data on sperm density of semen (Table-2), it revealed that the results were non-significant due to seasons and months. Between the bulls, the result was statistically significant. In rainy season the sperm density was affected which might be due to high atmospheric humidity as reported by Sengupta *et al.* (1963). These results were at par with the findings of Kodagali (*loc. cit.*) in Khillar bulls and Tomar *et al.* (1966) in buffalo bulls, but differs from the findings of Kushwaha *et al.*

TABLE 3: Average values of semen quantity and quality in different months.

Months	Volume (ml)	Density (D)	Mass activity (+)	Motility	
				After dilution & before freezing (%)	After freezing (%)
January	5.42±0.42	3.23±0.08	3.36±0.08	81.31±0.72**	51.35±1.32**
February	5.45±0.57	3.29±0.08	3.22±0.08	77.36±0.80	41.60±2.55
March	5.08±0.43	3.06±0.10	2.84±0.11	75.78±0.70	39.03±2.40
April	5.39±0.39	2.81±0.10	2.98±0.09	74.14±1.66	42.32±1.82
May	5.53±0.26	2.99±0.08	2.93±0.07	73.79±0.47	43.27±0.92
June	5.34±0.39	3.00±0.09	2.90±0.12	77.10±0.47	44.76±2.59
July	6.03±0.47	3.08±0.11	3.05±0.13	77.22±1.35	45.52±2.09
August	4.74±0.21	2.96±0.09	3.15±0.08	75.29±0.67	44.52±1.58
September	5.44±0.46	2.92±0.09	2.96±0.08	75.93±0.90	41.34±1.86
October	5.02±0.35	2.94±0.16	2.99±0.10	77.22±1.10	41.93±2.46
November	6.28±0.58	3.01±0.12	3.03±0.10	79.90±1.30**	51.68±1.71**
December	5.39±0.39	3.17±0.10	3.11±0.15	80.93±0.86**	48.14±2.37**

** Significant at 0.01 level

(*loc. cit.*), Roy (1958), Patel (*loc. cit.*), Tomer *et al.* (*loc. cit.*) and Bhosrekar (*loc. cit.*) in Murrah buffaloes. This might be attributed to breed different and regional climatic conditions.

The initial motility (mass activity) of the semen in different seasons and months was statistically non-significant. There was highly significant difference amongst the bulls in mass activity of the semen. The moderate atmospheric temperature and humidity in winter season could be the reason to get good initial activity. These results were in accordance with the findings of Kodagali (*loc. cit.*) in Khillar bulls but differs with the findings of Kushwaha *et al.* (*loc. cit.*), Sengupta *et al.* (*loc. cit.*) Gill *et al.* (*loc. cit.*) and Bhosrekar (*loc. cit.*) in Murrah buffaloes. It might be due to change in atmospheric temperature, humidity and air movement of different regions, as reported by Kaleff (1942) and Malkani (1954) in Murrah buffaloes.

The difference in sperm motility after dilution but before and after freezing was highly significant being highest in winter season. But no significant difference between rainy and summr season was observed. Between months also, the values differred highly significantly. In January the quality was superior and in March the quality was affected. There was no significant difference amongst the bulls. The moderate climatic conditions in winter over the other two seasons could be the reason to get good quality of frozen semen. These results coroborated the findings of Shukla and Bhattacharya (1952), Mukherjee and Bhattachraya (*loc. cit.*), Kodagali (*loc. cit*) and Amarjeet Singh *et al.* (1979) in bulls, rams and bucks. But it was contrary to the findings of Malkani (*loc. cit.*) Kushwaha *et al.* (*loc. cit.*), Roy (*loc. cit.*), Majeed *et al.* (1961); Sengupta *et al.* (*loc. cit.*), Gill *et al.* (*loc. cit*), Sidhu *et al.* (1979) and Bhosrekar (*loc. cit.*) in Surti, Murrah and Nili-Ravi buffalo bulls. This could be due to change in climatic conditions of the region. The buffalo bull is very sensitive to the extreme cold and heat and it cannot adopt itself to colder climate like cattle and therefore the

possible decline in semen quality due to seasonal variations was observed by Kaleff (*loc. cit.*). Also the thermo-regulatory mechanism is not so well developed in buffaloes as in other species of farm animals like bulls, rams and bucks, as reported by Kushwaha *et al.* (*loc. cit.*).

On the perusal of these observations, it was concluded that, there was no seasonal effect on semen volume, colour and consistency, sperm density and initial activity, but the improvement was observed in winter over the other two seasons. The good quality of frozen semen was obtained in winter season and January. The quality was affected in summer and March. Thus the atmospheric temperature, humidity and wind speed might play major role in maintaining semen quality in Surti buffalo bulls under Dharwad climatic conditions.

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Factors Associated With Dystocia In Crossbred Cattle: A Study Of 112 Cases

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ABSTRACT

112 cases of dystocia in crossbreds reported between July 1983 to June 1985 were included in this study and factors involved were investigated. It was observed that uterine and birth canal defects were higher (45.5%) as compared to foetal alignment defects (37.5 %). Pluriparous cows suffered more with alignment defects and defects of contraction forces as compared to the primipara in which the major problem was the defect of passage. 81.35% of the foetuses were in anterior presentation and the rest were posteriorly presented. In anterior presentation, head deviation was most common (60.42%) followed by limb deviations (39.58%). Further more, the incidence of head and limb deviations did not vary much in pluripara but in primipara the incidence of head deviation was much higher (71.43%) as compared to limb deviation (28.57). With regard to parity, it was found that highest incidence of dystocia (46.43%) occurred in primipara which decreased with subsequent parturitions. Incidence of dystocia in dams increased significantly ($p<0.01$) when carrying a male foetus in-utero as compared to female foetus and the mortality rate was also observed to be significantly high ($P<0.05$) amongst male foetuses.

* * *

Difficulty in parturition is one of the common obstetrical problems affecting cattle. It causes concern to the clinician and owner alike because the condition may be fatal for the dam and/or foetus and the future reproductive and productive ability of the affected animal remains in jeopardy. Dystocia results due to defects in power (uterine and abdominal contractions), passage (birth canal) or the carriage (foetus) or a combination of the above mentioned defects. For instituting proper and effective therapeutic measures and assistance it is imperative for the clinicians that the immediate cause of dystocia is detected. Knowledge of common factors associated with dystocia in a particular area may be helpful in order to undertake preventive measures as well. During the present investigation the incidence of different types of defects involving power, passage and carriage leading to dystocia in crossbreds has been worked out.

Materials and Methods

One hundred and twelve cases of difficult parturitions in crossbred cattle attended during the period from July 1983 to June 1985 around Ranchi were included in this study. The crossbreds were of different grades of Friesian X Local, stall fed with satisfactory general health conditions. A case was considered to be of dystocia when the first or second stage of parturition was markedly

TABLE 1. Maternal and Foetal Defects Leading to Dystocia in Cows and Heifers.

Defects	Cows		Heifers		Total	
	No.	Per cent	No.	Per cent	No.	Per cent
Uterine and birth canal defects	19	16.9	32	28.6	51	45.5
Alignment defects	28	24.1	14	12.5	42	37.5
Combined	11	9.8	6	5.3	17	15.2
Teratological	2	1.8	—	—	2	1.8

prolonged and the dam was not able to deliver the foetus spontaneously (Roberts, 1971). Complete history of the case was recorded regarding the date of last A.I., time of initiation of parturition, symptoms and appearance of water bag, earlier calvings etc. Thorough vaginal examination was conducted to ascertain the immediate cause of dystocia. Foetal misalignments were corrected by mutation and forced traction was applied to deliver the calf after proper lubrication. The quantum of traction depended upon the requirement for effective delivery.

Results and Discussion

The incidence of immediate causes of dystocia has been presented in table 1. It is apparent therein that the percentage of uterine and birth canal defects was higher (45.5%) in comparison to alignment defects (37.5%). It was further observed that the incidence of alignment defects was higher in pluripara (24.1%) than in primipara (12.5%), while uterine and birth canal defects were higher in the later (28.6%) as compared to pluripara (16.9%). The uterine and birth canal defects were classified into two categories viz., defects in power (uterine and abdominal contractions) and defects in passage (constriction of birth canal). It is evident from table 2 that in cows the incidence of defects in power (torsion of uterus, 7.35% and uterine inertia, 23.52%) was higher as compared to the defects of passage which was very high in primipara (incomplete relaxation of birth canal, 45.58% and inadequate pelvis, 7.35%). Sloss and Johnston (1967) recorded the highest incidence of failure of cervix and vagina to dilate completely followed by uterine inertia. Roberts (1971) described that dystocia due to constriction of the vulva and vestibule might be due to chronic diseases, poor nutrition, abortion or premature calving. Rice and Wiltbank (1972) also observed vulval stenosis in 54.5% cases of dystocia which is in agreement with the present findings. Friedli (1965), Sloss (1970) and Rice and Wiltbank (1972) reported that the incidence of vaginal and vulval constriction varied from 1% to 28% of all

TABLE 2. Incidence of Uterine and Birth Canal Defects Causing Dystocia.

Sr. No.	Defects	Cows		Heifers		Total	
		No.	Per cent	No.	Per cent	No.	Per cent
1.	Torsion of uterus	5	7.35	—	—	5	7.35
2.	Inertia	16	23.52	2	2.94	18	26.47
3.	Incomplete relaxation of birth canal	7	10.29	31	45.58	38	55.88
4.	Inadequate pelvis	—	—	5	7.35	5	7.35
5.	Uterine rupture	—	—	2	2.94	2	2.94

TABLE 3. Incidence of Different Types of Foetal Misalignment Causing Dystocia.

Sr. No.	Misalignment types	Cows		Heifers		Total	
		No.	Per cent	No.	Per cent	No.	Per cent
1.	Anterior Presentation:						
	I. Carpal flexion						
	Unilateral	1	1.69	1	1.69	2	3.38
	Bilateral	—	—	3	5.08	3	5.08
	II. Shoulder flexion						
	Unilateral	4	6.77	1	1.69	5	8.47
	Bilateral	6	10.16	1	1.69	7	11.86
	III. Dog sitting posture	2	3.38	—	—	2	3.38
	IV. Head deviation						
	Left lateral	7	11.86	10	16.94	17	28.81
	Right lateral	4	6.77	1	1.69	5	8.47
	Downward	3	5.08	4	6.77	7	11.86
	V. Total	27	77.14	21	87.5	48	81.35
2.	Posterior presentation:						
	I. Extended posture	4	6.77	2	3.38	6	10.16
	II. Bilateral hock flexion	2	3.38	—	—	2	3.38
	III. Bilaterlal hip flexion	2	3.38	1	1.69	3	5.08
	IV. Total	8	22.86	3	12.5	11	18.65
	V. Overall (1+2)	(35)		(24)		(59)	
3.	Total limb deviations	13	48.15	6	28.57	19	39.58
4.	Total head deviations	14	51.85	15	71.43	29	60.42
5.	Overall (3+4)	(27)		(21)		(48)	

Values in parentheses indicate number of cases in that particular group.

dystocias and was most frequently observed in older primiparous cattle. The incidence also depended upon the geographical region and seasonal conditions. Malinowski *et al.* (1983) reviewed that maternal factors leading to dystocia included narrow and dry passages and inadequate uterine contractions.

Incidence of different types of foetal misalignments causing dystocia has been presented in table 3. It is evident that 81.35% of the foetuses were in anterior presentation (48/59) while only 18.65% (11/59) were in posterior presentation. Foetal head was most commonly found to be deviated (60.42%) as compared to foetal limbs (39.58%). The incidence of left lateral deviation of head was observed to be the highest (28.81%) followed by downward deviation (11.86%) and right lateral deviation (8.47%). In cows there was not marked difference between the incidence of head or limb deviations (48.15% vs 51.85%). Whereas in heifers the incidence of head deviation was higher as compared to limb deviation (71.43% vs 28.57%). Out of 11 cases of posterior presentation, 6 foetuses (10.16%) had extended posture and 5 had flexed hind limbs (5.08% bilateral hip flexion and 3.38% bilateral hock flexion).

Sloss and Johnston (1967) observed that displacement of foetal head and/or limbs was one of the important causes of foetal dystocia besides other abnormalities, while Sloss (1970) and Laster *et al.* (1973) observed that postural abnormality caused 20% of all dystocias. Postural

TABLE 4. Incidence of Dystocia in Relation to Parity.

Parity status	No. of animals	Percentage
First calver	52	46.43
Second calver	12	10.71
Third	24	21.43
Fourth calver	14	12.50
Fifth calver	8	7.14
Sixth calver	2	1.78

defect leading to dystocia was also recorded by Raman and Bawa (1977). Mutiga *et al.* (1981) recorded misalignment defects in 38 foetuses out of which 15 were lateral head deviations, 10 had forelimb flexions and 13 foetuses were in posterior presentation. Malinowski *et al.* (1983) reviewed foetal factors causing dystocia and observed that mal-posture was one of the important factors. Sloss and Dufty (1980) opined that reflex response of the foetus to the myometrial contractions was necessary for adopting correct position and posture. Friedli (1965) concluded that incorrect posture occurred due to weak foetuses which could not extend their limbs. It was also noted that incorrect alignment always followed decreased foetal movements during late gestation and on this basis Fraser (1977) hypothesized that lack of foetal exercise might lead to diminished degree of muscular competence of the foetus for adopting correct posture. Dufty and Sloss (1977) observed that experimental anoxia of bovine foetus in-utero led to varying degrees of activity ranging from slight muscle tremors to vigorous erratic movements. It appeared to them that faulty alignment might arise when delivery was delayed and placental separation occurred leading to foetal hypoxia. Whereas, Dufty (1972*b*) opined that vulval or vaginal constrictions might lead to passive deflection of neck or limbs. Arthur (1966) and Uwaland (1976) reported that birth difficulty in cow developed 4 to 7 times more frequently when the foetus was in posterior presention than in anterior presentation.

Highest incidence of dystocia (52/112) was recorded in primiparous animals (Table 4). Friedli (1965), Sloss (1970), Balika (1965) and Smith *et al.* (1976) observed that the incidence of dystocia may reach 71% in heifers less than 2 years of age. They further observed that the incidence decreased to 66% and 44% in 2 and 3 years old primipara and ranged from 13 to 20 percent in multiparous cows. Rutter *et al.* (1983) also observed that 31.1% and 15.0% of cattle experienced calving difficulty at the first and second parturition respectively, while Verma *et al.* (1983) recorded a high incidence of dystocia at first parturition.

The results on sex ratio and mortality rate of foetuses associated with dystocia have been presented in table 5. Incidence of dystocia was significantly higher

TABLE 5. Sex Ratio and Mortality Rate of Foetus in Cases of Dystocia.

Sex of foetus	Live foetus		Dead foetus		Total	
	No.	Per cent	No.	Per cent	No.	Per cent
Male	35	31.81	33	30.00	68	61.81
Female	33	30.00	9	8.18	42	38.18
X^2 Value	—		13.71**		6.14*	

** $P < 0.01$ * $P < 0.05$

($P<0.01$) in dams carrying male foetuses as compared to those carrying female foetuses (61.81% vs 38.18%). It was further observed that the foetal mortality rate was significantly higher ($P<0.05$) in dystocia involving a male foetus (30.0%) as compared to female foetus (8.18%). Lasley *et al.* (1961) and Ellis *et al.* (1965) reported that birth weight of male foetuses was generally higher than their female counterparts. Several other workers have observed that incidence of dystocia increased with male foetuses (Vandeplassche *et al.*, 1965; Friedli, 1965; Dreyer and Smidt, 1966; Smidt *et al.*, 1978 and Malinowski *et al.*, 1983). A high incidence of dystocia with male foetuses in-utero has been reported to be due to their heavier weight and greater breadth (Laster *et al.*, 1973). However, Verma *et al.* (1983) reported a male to female foetus ratio of 51.8: 48.2 percent associated with dystocia. In one study, calves weighing 45.5 Kg or more were frequently found to be associated with dystocia during 1st and 2nd parturitions in dairy cows (Rutter *et al.*, 1983).

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Biochemical Profiles Of Repeat Breeding Crossbred Cattle In Relation To Different Phases Of Oestrous Cycle

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ABSTRACT

Biochemical profile with regards to trace minerals, blood glucose and blood urea level in normal cyclic and repeat breeding crossbred cows was studied. The results revealed that plasma trace mineral level were significantly lower in repeat breeder cows except zinc. Blood glucose was significantly higher in early phase of oestrous cycle. Blood urea was significantly higher in early phase of reproductive cycle thereafter it lowered.

* * *

A repeat breeding cow is one which has normal reproductive tract with normal oestrous cycle but which does not settle even to repeated services by fertile bull or inseminated by a good sample of semen. Repeat breeding is one of the major gynaecological problems lengthening the service period and affecting economics of milk production in dairy animals. The prevalence of this condition in crossbred cattle have extensively been reviewed by Casida (1961) and Kodagali (1981).

Information regarding biochemical attributes during different phases of reproductive cycle in repeat breeding cross-bred seems to be scanty. Although there are dangers in using single blood concentration to assess the metabolic state of an animal (Armstrong *et al.*, 1961), it was felt that the biochemical profile test if successful present an opportunity for the clinician to make a useful contribution towards solution of repeat breeding. In this paper an attempt has been made to study the level of plasma trace minerals viz., Ferrous (Fe), Mangenese (Mn), Zinc (Zn) and Copper (Cu), blood glucose and blood urea in relation to different phases of oestrous cycle of repeat breeding crossbred cows.

Materials and Methods

The study was conducted on 21 crossbred cattle of Livestock Research Station of Gujarat Agricultural University, Anand. The animals were raised under good management practice and subjected to thorough gynaecological examination for the presence of grossly palpable genital abnormalities and subclinical infection of genitalia. Previous and post breeding particulars were also recorded.

Six animals exhibiting physiological oestrous evidenced by the presence of mature graafian follicle in the ovary and showing regular conception formed the control group. Sixteen repeat breeding animals included that group of animals which were bred for more than five times in successive oestrous with fertile bull semen but failed to conceive.

TABLE 1: Blood plasma trace mineral, blood glucose and blood urea level in control and repeat breeding crossbred cows.

Reproductive phase	Fe (µg/ml)		Mn (µg/ml)		Zn (µg/ml)		Cu (µg/ml)		Blood glucose (mg%)		Blood urea(mg%)	
	Control (6)	Repeat breeder (15)	Control (6)	Repeat breeder (15)	Control (6)	Repeat breeder (15)	Control (6)	Repeat breeder (15)	Control (6)	Repeat breader (15)	Control (6)	Repeat breeder (15)
Oestrous phase (O day)	22.56 ±2.22	17.06* ±1.38	0.58 ±0.08	0.19** ±0.03	8.33 ±1.79	11.90 ±1.29	1.89 ±0.25	0.99** ±0.13	68.84 ±10.11	97.73* ±9.36	18.80 ±1.51	28.88** ±1.89
Early Luteal phase (4th day)	23.63 ±2.00	17.08* ±1.47	0.53 ±0.15	0.19* ±0.04	6.52 ±1.00	9.20 ±1.68	1.95 ±0.22	1.04** ±0.16	52.45 ±5.16	71.89* ±8.33	16.34 ±3.05	27.68* ±2.62
Mid Luteal phase (11th day)	23.94 ±2.57	16.77* ±1.50	0.48 ±0.11	0.18** ±0.03	8.55 ±2.15	6.81 ±0.97	1.82 ±0.22	0.96 ±0.16	60.90 ±4.58	62.78 ±4.72	22.39 ±3.42	19.25 ±1.91
Early Follicular phase (16th day)	24.33 ±1.71	16.52* ±2.02	0.56 ±0.13	0.20** ±0.04	4.45 ±0.94	9.48* ±1.33	1.82 ±0.29	0.91** ±0.15	55.55 ±4.88	57.06 ±4.21	20.00 ±2.47	17.41 ±2.61

Figures in parentheses indicate the number of animals.

* Significant at 5%. ** Significant at 1%.

Blood samples from each animal were collected from the jugular vein on the day of oestrous phase (0 day), early luteal phase (4th day), mid luteal phase (11th day) and early follicular phase (16th day) of reproductive cycle for the estimation of plasma trace minerals as described by Manickam *et al.* (1977), blood glucose and blood urea as per the method described by Hawk *et al.* (1954).

Results and Discussion

The blood plasma levels of trace minerals, blood glucose and blood urea in repeat breeding and control cows during different phases of oestrous cycle are shown in table 1. The blood plasma Fe content (μg/ml) of control cows varied from 22.56 ± 2.22 on the day of oestrous phase to 24.33 ± 1.71 at early follicular phase, but that in the repeat breeder was 17.06 ± 1.38 on day of oestrous phase to 16.52 ± 2.02 at early follicular phase of oestrous cycle. The observed difference between the groups was significant ($P<0.05$) at all phases of reproductive cycle.

The blood plasma Mn level (μg/ml) of control cows ranged from 0.58 ± 0.08 at oestrous phase to 0.56 ± 0.13 at early follicular phase, while that of the repeat breeder was 0.19 ± 0.03 at oestrous phase to 0.20 ± 0.04 at early follicular phase. The difference in Mn level was highly significant ($P<0.01$) at 0, 11th and 16th day and ($P<0.05$) at 4th day of reproductive cycle.

The blood plasma Zn (μg/ml) level of normal cows ranged from 4.45 ± 0.94 at follicular phase to 8.55 ± 2.15 at mid luteal phase, while that of the repeat breeder it was 6.81 ± 0.97 at mid luteal phase to 11.90 ± 1.29 at oestrous phase. The difference observed between the groups was significant ($P<0.05$) only at early follicular phase.

The blood plasma Cu (μg/ml) level of control cows varied from 1.82 ± 0.29 at early follicular phase to 1.95 ± 0.22 at early luteal phase of oestrous cycle, but for repeat breeder it was from 0.91 ± 0.15 at early follicular phase to 1.04 ± 0.16 at early luteal phase of reproductive cycle. The difference was significant ($P<0.01$) with respect to repeat breeding animals over the periods specified.

Blood glucose (mg%) level of control group varied from 52.45 ± 5.16 at early luteal phase to 68.84 ± 10.11 at oestrous phase while in repeat breeding cows it ranged from 57.06 ± 4.21 at follicular phase to 97.73 ± 9.36 at oestrous phase. The difference between the groups was significant ($P<0.05$) at oestrous phase and early luteal phase but not at mid luteal and early follicular phase of oestrous cycle.

Blood urea (mg%) content of regular breeding cows ranged from 16.34 ± 3.05 at early luteal phase to 22.39 ± 3.42 at mid luteal phase, but that of repeat breeding cows varied from 17.41 ± 2.61 at follicular phase to 28.88 ± 1.87 at oestrous phase of oestrous cycle. The difference between the groups was significant at oestrous phase ($P<0.01$) and early luteal phase ($P<0.05$).

Trace elements may function as co-factors, activator of enzymes or stabilizing of secondary molar structure. Hidiroglou (1979) reviewed the trace mineral status of ruminants in relation to their fertility. The reproductive failures are induced in experimental trace mineral deficiency. The present studies revealed that blood plasma level of trace minerals remained significantly higher in control cows than the repeat breeding cows. This finding is in close approximation with that of Manickam *et al.* (1977). In the recent studies with repeat breeding buffaloes,

however, this trend was not established (Jain and Madan, 1984). This is again showing species difference on trace mineral status of cows and buffaloes. Plasma Zn content of repeat breeder cows was higher than the control.

Hypocuprosis in cattle is associated with reproductive disorders including fertility failure (Mahadevan and Zubairy, 1969). Metabolism of Copper seems to be altered in the repeat breeding cows. It is not clear whether Cu status alone is influencing the reproductive status of the ruminants or it is the definite ratio between different trace minerals which may be deciding factors in regulating fertility. Since both the groups were kept in same management and nutritional status the altered plasma level of trace mineral in repeat breeding cows can be explained only on the basis of difference in absorption and catabolism rate of these minerals caused by specific hormone status.

The blood sugar levels of repeat breeding cows were significantly higher ($P<0.05$) during oestrous and early luteal phase when compared with control cows. The other stages of oestrous cycle did not reveal any significant changes. The hypothesis that hypoglycemia may affect the fertility of cows by causing disturbance in hypothalamic-hypophyseal axis (Howland *et al.*, 1966) seems to be conclusive since the cows keeping blood sugar level lower than 25 mg % or injection of insulin adversely affected the fertility of these animals (Masson *et al.*, 1973). This aspect is related to low level of energy intake because the level of feeding and fluctuation in feed supplies probably account for the largest variation among herds and even among individuals in the same herd (McDowell, 1972). The present study revealed that the repeat breeding cows persistantly kept high blood sugar level especially during important phases such as early follicular and early luteal phase. Sharma *et al.* (1984) demonstrated slight hypoglycemia in repeat breeding cows. However, Prasad *et al.* (1984) reported a wide range (32-102 mg %) of blood sugar in anoestrous cows. The repeat breeding animals seems to be either fed with high energy levels or are in specific metabolic stress during follicular and luteal phases. The hyperglycemia due to high energy intake or diabetes melitus in human subjects have also shown the importance in fertility. It is essential to prove the hypothesis about hyperglycemia affecting the fertility of cows either by infusing glucose or injecting glucocorticoids during oestrous and early luteal phases. The effect of hyperglycemia on the uterine tonicity and myometrial contractibility needs to be studied in detail.

The blood urea concentration in oestrous and early luteal phase seems to be elevated significantly when compared with control cows. This finding is parallel with that of blood sugar level studies. However, the earlier studies on blood urea in repeat breeding cows did not show this trend (Rowland *et al.*, 1977). The hypothesis that high energy protein intake or metabolic stress may lead not only to hyperglycemia and high blood urea level seems to be quite teneble. However, the fertility studies with high protein or infusion of urea need to be made to establish this hypothesis.

The above findings conclude that blood metabolites and trace mineral level get disturbed in repeat breeder cows. This may be due to hormonal imbalance or latent infection. The correction of these parametric changes back to normal may settle the repeat breeders towards pregnancy.

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Cytohormonal Indices In Different Reproductive Conditions In Bovines

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ABSTRACT

Cytohormonal indices can be beneficially employed in assessing the hormonal status in bovines. Maturation index and superficial cell index were considered to be easy to work out and accurate enough to evaluate the hormonal status in different reproductive conditions. Karyopyknotic index was observed to be more accurate but difficult to work out as it involves careful identification of cells with pyknotic nuclei and differentiate them from those with vesicular nuclei.

* * *

Parabasal, intermediate, superficial and cornified cells are the exfoliated cells normally noticed in a vaginal smear. The percentage of incidence of these cells was employed as cytohormonal indices in canine (Schutte, 1967) in women (Naib, 1976) and in camel (Banerjee *et al.*, 1982). In bovines there are reports on vaginal cytology (Hussain and Khan, 1979; Rao and Rao, 1982 and Siddique *et al.*, 1984) but no attempt has been made to evaluate the vaginal smear based on cytohormonal indices.

The present investigation was taken up to work out the cytohormonal indices in cows and buffaloes in different reproductive conditions.

Materials and Methods

Cows and she buffaloes of different parity that attended the Artificial Insemination centre and Gynaecology ward of the Veterinary Hospital, Madras Veterinary College were utilised for this study. Based on detailed gynaecological examination, clinical signs and the history given by the owner 125 animals included in this study were categorised into 1. Oestrus, 2. Dioestrus, 3. Pregnant (early, mid and late), 4. Anoestrus, 5. Endometritis and 6. Cystic ovarian groups.

Disposable plastic tongue cleaner held in the form of a loop in a long artery forcep was used to gently scrape the dorsolateral part of the anterior vaginal mucosa. By recto-vaginal method the location in the vagina was kept almost uniform for smear collection. The scrapings collected on the plastic loop was spread on a slide and fixed immediately with ether-alcohol mixture (1:1) for 15 minutes. The fixed smear was then stained with Shorr's stain (Bancroft and Stevens, 1977) cleared in xylol and mounted in DPX for detailed cytohormonal analysis.

Cytohormonal Indices:

i) *Maturation index* (MI): The percentage of parabasal, intermediate and superficial cells were presented as a three part ratio with the parabasal cells stated

TABLE 1. Cytohormonal Indices in Different Reproductive Conditions in Cows and Buffaloes

Reproductive conditions	Maturation Index		Karyopyknotic Index		Superficial Cell Index		Maturation value	
	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo
Oestrum (30)	31/22/47	31/34/35	42	35	55	41	57	47
Dioestrum (25)	37/43/20	31/46/22	20	28	35	40	45	42
Anoestrum (26)	60/20/10	52/28/20	18	13	27	29	27	28
Pregnant:								
Early (10)	43/34/23	53/31/16	16	18	23	15	40	31
Mid (10)	41/33/26	50/29/21	17	20	22	25	34	37
Late (10)	44/24/32	50/20/30	20	20	28	28	39	38
Endometritis (10)	28/23/49	40/26/34	43	41	48	45	52	47
Cystic ovary (4)	20/32/48	—	48	—	61	—	59	—

Figures in parantheses indicate number of animals/observations.

first, the intermediate cell second and the superficial cells third.

ii) *Karyopyknotic Index (KPI)*: The percentage of squamous epithelial cells with sharp, squared-off cytoplasmic edges and with pyknotic nuclei was given in relation to all other mature squamous epithelial cells that possess vesicular nuclei regardless of the type of staining quality. The parabasal cells were not counted.

iii) *Superficial cells index (SCI)*: Only the superficial cells, regardless of the staining quality of their cytoplasm and nuclear characteristics were evaluated and given in relation to all other squamous cells including parabasal cells.

iv) *Maturation value (MV)*: Each parabasal cells was counted as O, each intermediate cells was counted as 0.5 and each superficial cells as 1. The addition of all the values given to the first 100 epithelial cells was recorded.

Results and Discussion

Cytohormonal indices in different reproductive conditions in cows and buffaloes is presented in table 1.

According to Naib (1976) in Maturation Index (MI) shift to the right was due to the effect of oestrogen, shift to the middle was due to progesterone and shift to the left indicate atrophic changes. In the present study MI of 31-22-47 in cow clearly indicated shift to the right but in buffaloes the value was 31-34-35 with no clear evidence of shift to the right. This may be due to the reduced influence of oestrogen on the vaginal epithelium in buffaloes. In both cows and buffaloes during dioestrum there was shift to the middle suggesting increasing progesterone effect. In anoestrum the shift to the left was evident. This could be due to atrophic changes of vaginal epithelium in the absence of oestrogen and progesterone hormones.

In pregnant animals shift to the left was evident in both cows and buffaloes. The reason for this change eventhough the vagina was under the influence of progesterone from corpus luteum of pregnancy could not be explained based on the available data. According to Hemkemeyer (1976) sows were pregnant if the vaginal smear showed more than 85 per cent basal cells or 80 per cent basal and 10 per cent parabasal cells. It

was interesting to note that the proportion of superficial cells tend to increase from 23 at early to 26 at middle and then to 32 at late pregnancy. The increase could be due to the effect of oestrogen secreted at late stage of pregnancy (McDonald, 1980). In endometritis and cystic ovarian condition the maturation index was similar to that of oestrus and did not show any specific variation because of the pathological condition of reproductive organs. Of the conditions studied, shift to the right was maximum in cystic ovarian condition which could be due to persistant oestrogen effect on the vaginal eithelium.

The mean karyopyknotic index(KPI) value in oestrus cows was 42 in contrast to 35 in buffaloes. In dioestrus, anoestrus and during pregnancy the KPI value continued to be very low in both cows and buffaloes. Since smears were collected during oestrus in endometritis and cystic ovarian conditions the KPI value was almost similar to that of oestrus. However, the maximum value of 48 was noticed in cystic ovarian condition. Many authors have reported on the cornification index which was closely related with KPI. Bader *et al.* (1978) reported in mare rapid rise in cornified cells to a maximum of 90-100 percent close to clinically palpable ovulation. In bitches the peak of the cornification index was noticed at the time of ovulation (Taradach, 1980). In cows, Rao and Rao (1982) observed that the cornification was slight at the onset of oestrus, moderate during late oestrus and intense after ovulation. Decrease in KPI noticed during dioestrus and pregnancy agreed with the report of Niaraki *et al.* (1981) who considered the decrease in KPI and increase in intermediate cells during mid cycle was due to progesterone effect.

The mean superficial cell index (SCI) in cow was 55 in oestrus in contrast to 35 and 27 in dioestrus and anoestrus conditions. The corresponding values in buffalo were 41, 40 and 29. During pregnancy the SCI ranged from 23 to 28. In buffaloes it was 15 to 28. The maximum value of 61 was noticed in cystic ovarian conditions. Schutte (1967) who studied SCI value in bitches noticed high value (70 per cent) during proestrus phase which remained high even during oestrus phase. He concluded that SCI value will be of greatest value in assessing the effect of hormonal therapy.

The maturation value showed the same trend of changes as the other cytohormonal indices in different reproductive conditions. Naib (1976) who has worked out maturation value in different conditions in human considered that this index was useful in providing hormonal evaluation.

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Serum Progesterone Levels In Cows Retaining Foetal Membranes

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ABSTRACT

The serum progesterone (P) levels were determined by radio-immunoassay on 260th and 270th days of gestation, on calving, and 12th hour, 1st, 3rd, 5th, and 10th days post-calving in 6 cows retaining fetal membranes (FMs) and 6 cows not retaining FMs. The mean serum P level in cows not retainning FMs continued to decline from 1.46 $\pm$0. 18 ng/ml on 260th day gestation to 0.19$\pm$0.09 ng/ml on 3rd day postpartum. In cows retaining FMs the mean P level on 260th day of gestation was 3.20$\pm$ 0.67 ng/ml which declined to 0.15 $\pm$ 0.2 ng/ml on 3rd day postpartum. The prepartum P levels, in general were found to be higher in cows retaining FMs than in the cows not retaining FMs. The possibility of this high P concentration in cows retaining FMs resulting in an asynchrony of hormonal mechanisms that normally synchronize parturition and expulsion of FMs is discussed.

* * *

Retention of fetal membranes is a common postpartum complication in bovine and results in great economic loss to the dairy industry unless treated promptly. The etiology and pathogenesis of retained fetal membranes is incompletely understood. The present study reports the serum progesterone levels in cows retaining fetal membranes.

Materials and Methods

Blood samples (20 ml) were collected from the jugular vein of 12 cows (6 retaining and 6 not retaining FMs) on 260th and 270th days of gestation, on calving, and 12th hours, 1st, 3rd, 5th and 10th days postpartum. The animals were in their 1st to 6th lactation during the study. All the animals included in the study were kept under good feeding and managerial condition. The serum was separated from the clotted blood samples by centrifugation and stored at — 20°C until used for progesterone estimation.

Serum progesterone was estimated by Radioimmunoassay (RIA) technique as described by Thorneycroft and Stone (1972) with minor modification. Aliquots of serum samples were extracted with 5 ml of diethyl ether on a vortex mixer for one minute. After extraction the aqueous phase was frozen and the ether extract was decanted in assay tubes. The extract was dried in water bath at 37°C. The dried contents were dissolved in 0.1 ml phosphate buffer saline (PBSG) con-

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TABLE 1. Serum progesterone level (ng/ml) in cows not retaining and retaining fetal membranes

Groups	Sr. No.	Animal No.	Gestation Length (days)	Gestation days 260th	Gestation days 270th	On calving	12th hour postpartum	Days postpartum 1	Days postpartum 3	Days postpartum 5	Days postpartum 10
Not retaining fetal membranes											
	1	RS—90	285	1.60	1.10	0.66	0.68	0.22	0.14	0.22	0.30
	2	RS—131	284	1.30	1.12	1.16	0.28	—	0.15	0.20	0.16
	3	RS—109	283	1.30	0.96	0.58	0.14	0.20	0.38	0.56	0.18
	4	AC—689	275	2.30	2.50	—	0.74	0.15	—	0.12	0.13
	5	RS—81	278	1.18	0.80	—	0.35	0.27	0.15	0.20	0.20
	6	RS—78	282	1.06	1.02	—	0.36	0.50	0.12	0.20	0.11
		Mean ± SE		1.46 ±0.18	1.25 ±0.25	0.80 ±0.18	0.43 ±0.10	0.27 ±0.06	0.19 ±0.09	0.25 ±0.07	0.18 ±0.03
Retaining fetal membranes											
	1	RS—70	273	3.50	1.90	2.05	1.60	1.30	0.21	0.20	0.14
	2	RS—64	280	—	0.90	—	—	0.36	0.15	0.46	0.20
	3	HF—9	270	1.30	—	—	0.85	0.24	0.18	0.16	0.10
	4	RS—140	277	3.60	—	—	0.30	0.40	0.08	0.14	0.12
	5	RS—47	277	4.40	—	0.54	0.56	0.26	0.14	0.21	0.14
	6	RS—126	280	—	—	0.51	0.31	0.40	0.16	0.10	0.16
		Mean±SE		3.20 ±0.67	1.40 ±0.50	1.03 ±0.51	0.72 ±0.24	0.49 ±0.16	0.15 ±0.02	0.21 ±0.05	0.14 ±0.01
		"t" values		3.0526*	0.2885	0.4259	1.2083	1.1579	1.000	0.4444	1.3333

* $P<0.05$

taining 0.1 M phosphate buffer, 0.9 per cent sodium chloride, 0.1 per cent sodium azide and 0.1 per cent gelatin (pH 7.0) followed by the addition of 0.1 ml antisera and 0.1 ml lebelled progesterone (approximately 10,000 cpm). The contents of each assay tube were mixed after each addition and then incubated overnight at 4°C. At the end of incubation, 0.5 ml dextran charcoal suspension (500 mg activated charcoal and 50 mg dextran T-70 in 100 ml ice cold PBSG) was added to each tube, vortexed for 10 seconds and allowed to stand in ice waterbath for 15 minutes. The tubes were centrifuged at 3,000 rpm for 15 minutes at 4°C in a refrigerated centrifuge. The supernatent was decanted in scintillation vials containing 1 ml of ethanol. To each vial, 10 ml of scintillation fluid (4.0 gm PPO and 0.1 gm Dimethyl POPOP dissolved in 1 litre of toluene) was added and the contents were mixed. The vials were counted for 5 minutes in Beckman LS-100-C liquid scintillation spectrometer. All the serum samples were processed in duplicate. The standards, blanks and serum pool of known progesterone content were also processed in duplicate, with each set of samples in the similar way. The concentration of progesterone in unknown samples were read out against interval standard curves.

The within assay variance for different ranges of progesterone was calculated from 3 triplicate determinations. The coefficient of variation (CV) was found to be 8.52 per cent. The between assay variance was calculated from 3 duplicate low serum progesterone pool (serum

from castrated male) and 3 duplicate high progesterone pool (serum from late gestation of cattle) and was found to be 17.19 per cent. The standard curve for progesterone was linear in the range of 25 to 1000 pg.

Results

The serum progesterone levels in 6 cows retaining and 6 cows not retaining their fetal membranes are presented in table 1.Although there were individual variations in progesterone levels on all the occasions of observation, it was noted that the mean serum progesterone levels continued to fall from 260th day of gestation till the 3rd day postpartum in both the group of cows retaining and not retaining fetal membranes. From 3rd day postpartum onwards the mean serum progesterone levels remained almost constant in both the group of animals till 10th day postpartum. The difference between the mean serum progesterone levels in cows retaining and not retaining their fetal membranes were statistically non significant on all the times of sampling except on 260th day of gestation when it was significantly higher ($P < 0.05$) in cows retaining their fetal membranes than in cows without fetal membranes retention.

Discussion

The mean serum progesterone levels in cows not retaining their fetal membranes started declining from 1.46 ± 0.18 ng/ml on 260th day of gestation to reach a level of 0.18 ± 0.03 ng/ml on 10th day postpartum. Donaldson *et al.* (1970), Roberstson *et al.* (1972) and Arije *et al.* (1974) also reported a similar trend in the progesterone concentration during the last 2 to 3 weeks before calving in cows. However, Smith *et al.* (1973) reported that the progesterone concentration remained high until 2 days before calving in cows. Although there were individual variations in serum progesterone levels of 6 cows not retaining fetal membranes, that were included in the present study, the general pattern in all the animals was similar.

The serum progesterone levels in the experimental cows during gestation were found to be much lower than those reported for exotic cows by Short (1958), Stabenfeldt *et al.* (1970), Arije *et al.* (1974). However, Agrawal *et al.* (1977) reported that the levels of progesterone during the luteal phase were much lower in Zebu cattle as compared to the levels in exotic breeds. The results of the present study in the prepartum animals are comparable with those reported by Agarwal *et al.* (1977) for luteal phase.

The serum progesterone levels in cows retaining their fetal membranes were found to be 3.20 ± 0.67 ng/ml on 260th day of gestation. The levels declined subsequently reaching 0.14 ± 0.01 ng/ml on 10th day postpartum. The serum progesterone level was significantly higher ($P < 0.05$) on 260th day gestation in cows retaining their fetal membranes than in the cows expelling their fetal membranes normally. However, the differences in the serum progesterone levels between the two groups of animals on subsequent days of sampling were not statistically significant. Edqvist *et al.* (1972) also did not find any significant difference in progesterone levels in cows retaining and not retaining fetal membranes on the second day postpartum. Similar results were also reported by Agthe and Kolm (1975).

The prepartum progesterone levels were found to be higher in cows retaining their fetal membranes than in the cows not retaining their fetal membranes. Similar results were also reported by

Chew *et al.* (1977 and 1978).

In the present study the serum oestradiol levels could not be estimated to find out the relationship between the oestradiol and progesterone levels in relation to retention of fetal membranes in cows. However, Chew *et al.* (1977) reported that the levels of oestradiol 17-β and prolactin were lower during the prepartum period in cows retaining fetal membranes than in cows not retaining their fetal membranes. These results along with the serum progesterone levels observed in the present study indicate that the increased progesterone, decreased oestradiol 17-β and possibly decreased prolactin in the blood of animals retaining their fetal membranes as compared to those not retaining fetal membranes may results in an asynchrony of hormonal mechanisms that normally synchronize parturition and expulsion of fetal membranes.

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Blood Serum Alkaline Phosphatase (AKP) And Peroxidase In Pregnant Surti Buffaloes

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ABSTRACT

Total of 24 pregnancies covering three successive parities of Surti buffaloes were studied. Starting from fertile heat though parturition and 2 hrs post-partum, 27 stages were marked for blood collection. Average activity for AKP and peroxidase was 14.53 K.A. Unit% and 3.68 OD/ml, respectively. Pregnant buffalo showed lower AKP and higher peroxidase activity compared to cycling heifers. The AKP activity showed remarkable fluctuations for certain stages of gestation. It was higher during early pregnancy (upto day 50th where it attained the highest, 18.34 ± 3.33 K.A. Unit %). For the major part of mid-gestation it fluctuated non-significantly and decreased towards the term. However, a noticeable fall was recorded between day 65 to 95. Peroxidase activity did not show any significant variation (fluctuated within the range of 2.42 to 4.20 OD/ml), for different stages of pregnancy.

* * *

Alkaline phosphatase activity is known to have a role in certain events of reproductive physiology (Mardoeh, 1971). Both AKP and peroxidase activity reflect thyroid activity. It is also known that peroxidase involves somewhere in the process of iodination (Vadodaria *et al.*, 1978). Estimation of these two enzymes in blood serum of pregnant buffalo over entire pregnancy is the first attempt. This study was conducted to know the basic levels of these two enzymes and their fluctuations for different stages of entire pregnancy which indirectly reflects the demand and utilization by the foetus, dam or both.

Materials and Methods

Total of 24 pregnancies of Surti breed buffalo were included covering three sucessive parities. All the animals were disease free and normal in their reproductive performance. Blood was collected from jugular vein at preplanned scheduled stages over the entire pregnancy (from fertile heat through parturition and 2 hrs post-partum). Frequency of collection was higher during early and late gestation, while mid-gestation had low frequency (Table 1). The serum was separated and analysed for alkaline phosphatase (King and Armstrong, 1934) and peroxidase (Machly, 1954). These data were statistically analysed to know the stage variation as per Steel and Torrie (1960).

Results

Average levels of alkaline phosphatase and peroxidase for different stages of gestation over three parities have heen given in Table 1.

TABLE 1: Average serum alkaline phosphatase (K.A. Unit/100 ml.) and Peroxidase (OD/ml.) for different stages of gestation over three parities (Mean ± S.E.).

Characters	H	2nd	3rd	5th	10th	15th	20th	25th	30th
AKP	14.09	17.60	15.47	16.24	18.03	16.02	17.20	16.81	15.84
	1.81	3.90	3.95	2.24	3.02	2.06	2.88	2.93	1.78
Peroxidase	3.50	3.88	4.20	3.35	3.70	3.16	3.83	3.57	3.78
	0.04	0.59	0.45	0.79	0.69	0.36	0.66	0.60	0.45
	35th	50th	65th	80th	95th	125th	155th	185th	215th
AKP	16.21	18.34	14.73	15.30	11.98	14.69	13.27	15.49	12.76
	3.92	3.33	3.55	2.24	1.90	3.85	3.02	1.97	2.08
Peroxidase	3.95	3.20	3.47	3.71	3.33	3.21	3.34	3.03	3.44
	0.37	0.52	0.40	0.52	0.29	0.37	0.79	0.61	0.60
	245th	260th	270th	275th	—15	—10	—5	AP	PP
AKP	14.56	13.29	14.19	13.73	12.93	12.13	11.42	11.43	14.44
	2.64	2.30	3.99	1.94	2.34	1.21	0.83	0.85	4.01
Peroxidase	3.44	3.40	3.23	3.59	3.23	3.36	3.14	2.42	4.71
	0.71	0.43	0.68	0.87	0.56	0.62	0.53	0.49	0.80

H = Fertile heat; —ve sign indicates days ante-partum
AP = 2hr. ante-partum; PP = 2hr. post-partum.

Alkaline Phosphatase: The estimate was considerably high and had increasing trend from fertile heat to day-50 of gestation where it attained the peak (18.34±3.33 K.A. Unit/100 ml, Table-1). Specifically the activity was high between day-15 to day-50. After that the level of AKP dropped down significantly ($P<0.0.5$) by day 90 (Table-1). After day-125 it was fluctuating with a remarkable decreasing trend after day-270. It elevated soon after parturition (Table-1).

Peroxidase: Peroxidase activity did not show any significant trend for any stage of gestation (Table-1). Overall average activity came out to be 3.68 OD/ml, which fluctuated within the range of 2.42 to 4.70 OD/ml. Towards the term (last 10 days of gestation, —Table-1) the activity decreased reaching lowest at 2 hr antepartum and it increased immediately after parturition. This difference was statistically significant ($P<0.05$).

Discussion

Study on buffalo heifers (cycling) reported an average of 27.72 KA Unit/100 ml for alkaline phosphatase (Vadodaria *et al.*, 1978) and that for peroxidase 1.65 OD/ml (Anon., 1976). The result of present study gave an average of 14.53 K.A. Unit/100 ml for AKP and 3.62 OD/ml for peroxidase. This reveals that pregnant buffaloes possess lower AKP and higher peroxidase activity compared to heifers.

The AKP activity was appreciably high during early gestation (fertile heat to Day 50) compared to rest of the gestation period. Within this, Day 15 to 50 had the highest (Table 1). This is the period of gestation wherein fertilization, downward movement of zygote along the Fallopian tubes, release of blastocyst, uterine development, implantation and chorionic development take place. These situation increases the demand of nutrients resulting higher metabolic rate in the dam which has

been justified by reflecting thyroid activity deal with basal metabolic rate (Pathak *et al.*, 1984). This gives a positive association between thyroid activity and serum AKP. Such association has been reported by Ghose *et al.*, (1974) in cattle and Vadodaria *et al.* (1978) in buffaloes. Serum peroxidase did not show any considerable fluctuation during this period except slight increase on Day 3 (Table - 1).

A considerable fall in serum AKP was recorded from Day 50 to Day 95 where it was the lowest (11.98 K.A. Unit/ 100 ml, Table-1) This is the period of growing foetus where bone formation is the vital feature as per Mc Donald (1980) for cattle. Hence the decrease in the level observed in the present study may be due to utilization of this enzyme based material for growing foetus. This probably indicates a role of maternal serum AKP on growing foetus.

No significant change in serum AKP or peroxidase has been recorded over major period of mid-gestation.

Serum AKP again decreased during last 15 days of gestation. Sharma and Luktuke (1981) in Murrah buffalo and Surynek and Tomsik (1977) in cattle also observed a fall in AKP activity in dams circulation. They also estimated the foetal AKP for that stages which was increasing. They opined that reduction in dam AKP may be due to its utilization by the "term-foetus" for its oesteoblastic activity. The same reason may hold true for Surti buffalo. Peroxidase activity was also decreasing for last 10 days but changes were non-significant (Table-1).

Both the estimate decreased at 2 hrs AP and increased at 2 hrs PP. The difference was statistically significant for both. This can be referred to as utilization for the process of parturition and demand for lactational physiology.

On individual observations it was noticed that one buffalo (No. 57) had very short gestation length (280 days) in second parity compared to average gestation length (307 days) as well as its first parity gestation length (330 days). Significant low level of AKP as recorded towards the term for this animal (range 3.54 to 6.68 K.A. Unit/100 ml) while other animals had normal gestation length (300 days or above) which at that stages (280 to 285th day of gestation) showed considerably higher level (range 10.00 to 25.27 KA Unit%). Apart form this, all other animals also showed decreasing trend towards the term. From these observations parturition can be predicted by the trend of AKP.

Practically very limited work has been done on these enzymes over entire pregnancy in buffalo, so these data may also serve as basic norms for this breed of buffalo.

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Andrological Investigations In Cross-bred Bulls With Testicular Hypoplasia

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ABSTRACT

Among nine cross-bred bulls under diagnostic and investigative andrological studies at the Department of Gynaecology & Obstetrics, Gujarat Veterinary College, Anand, two bulls were diagnosed as problem bulls. One bull was found to have complete bilateral testicular hypoplasia. The other had low germ cell resistance hypoplasia and subsequent testicular degeneration. Libido and serving behaviour were exceptionally normal in both these bulls. The confirmative diagnosis was based on clinical examinations, semen evaluations, study of seminal biochemical profiles, fertility results and testicular histo-pathology.

* * *

The advent of cross-breeding with exotic bulls in India has brought to light the possible existence of testicular hypoplasia in both exotic and cross-bred bulls. Intensive screening of apparently normal bulls ment for breeding, either natural or artificial, is therefore necessary to identify and eliminate affected bulls. The present paper places on record a detailed report of diagnostic and investigative andrology in two cross-bred bulls with testicular hypoplasia, out of nine cross-bred bulls examined for the purpose.

History and Clinical Andrological Examinations

Low Germ Cell Resistance Hypoplasia (LGCRH): Bull Rosy:

The history among 8 cross-bred bulls of 5-6 yrs age, investigated at the Department of Gynaecology, Gujarat Veterinary College, Anand for a period of one year, showed that one bull (Rosy 8-14; K×J) was giving consistently poor semen quality as regards to motility, live-dead and abnormal sperm count, low resistance to cold and hot shock tests, low freezability, high rejection rate of semen samples frozen and low fertility as compared to other seven bulls, since beginning. The bull had normal serving ability and serving behaviour.

Bilateral Total Testicular Hypoplasia: Bull Deolo:

Another cross-bred bull (Deolo-K×HF ×HF — 75%) approximately 3 years of age, belonging to St. Xavier's Dairy Farm, Mogar (Anand) under natural service programme, was reported to be infertile. The history showed complete failure of conception among 35 cows bred during last one year. However, libido and serving behaviour were exceptionally normal.

Clinical examination (Rosenberger, 1979) revealed that both the testicles were smaller in size (Fig-1). The libido and sexual behaviour were excellent but with watery semen and practically

Fig. 1: Bilateral testicular hypoplasia-bull Deolo: note grossly small size testes and very small scrotum.

no spermatozoa. On rectal exploration, seminal vesicles were smaller in size. The semen was collected by artificial vagina with due asepsis under twice a week collection schedule for a period of 3 weeks and the detailed findings were compared with the average of 7 normal bulls (Table-1).

Histo-pathological studies of testicles were made at the end after open method of castration in both the affected bulls.

Results and Discussion

Clinical Andrological Studies:

Clinical andrological examination revealed excellent libido, serving behaviour and normal reaction time in both the affected bulls. However, testicular measurements and scrotal circumference were considerably lower in bull Deolo. These findings are in close agreement with those of Blockey (1975) who reported no direct relation between libido and seminal characters or scrotal circumference in bulls. Mickelsen and Paisley (1981) classified the potential of breeding bulls to be zero for those having scrotal circumference below 30 cms. Bartlett (1984) mentioned that the questionable or culled bulls had significantly lower scrotal circumference. These findings closely agreed with the present finding in bull Deolo. However, Jakubiec (1984) stated that scrotal circumference and testicular size provided a good fertility index.

Semen evaluation and Fertility:

Semen evaluation in bull Deolo revealed significantly low ejaculate volume, watery thin consistency, nil motility, very low sperm concentration specially with haemocytometric method, very high dead and abnormal sperm per cent with maximum head abnormalities and proximal cytoplasmic droplets, very low live sperm per cent, 100% dead sperm due to cold shock test and 94.57% dead sperm due to hot shock test (Table 1). In addition to high abnormal sperms a number of seminiferous epithelium like degenerated spermatocytes and spermatids with few giant cells were also observed in the semen centrifugate. The history in the bull indicated nil conception rate among 35 cows served during one year period. These findings closely agreed with those of Kaikini *et al.* (1978) who reported bilateral total testicular hypoplasia in a Holstein Friesian bull with complete azospermia.

TABLE 1. Comparison of sexual behaviour, testicular biometry, semen characteristics and seminal biochemical profiles in normal and hypoplastic bulls.

Attributes	Hypoplastic bulls		Normal bulls (7)
	Deolo-K×HF×HF: (6)	Rosy-K×J (24)	3 K×J & 4 K×HF (24×7=168)
I Sexual behaviour:			
Libido (0—3 scale)	2.83±0.29	2.13±0.07	2.24±0.04
Thrust (0—2 scale)	1.83±0.29	1.91±0.08	1.91±0.02
Reaction time (sec.)	105.00±29.25	86.67±8.94	125.15±11.88
II Testicular biometry:			
Scrotal circumference (cm)	25.67±0.82	35.83±0.20	36.93±0.72
Testicular volume (ml)	441.67±27.01	1233.33±20.42	1137.59±47.65
Testicular height (cm)	06.28±0.21	16.23±0.58	15.56±1.03
III Seminal characteristics:			
Ejaculate volume (ml)	2.92±0.52	6.79±0.41	7.09±0.20
Color & consistency (Scale 1-5)	1.50±0.52	4.33±0.09	4.44±0.06
Mass motility (0—4 scale)	Flat	2.85±0.26	3.68±0.08
Individual motility (%)	Zero	55.20±5.51	80.51±1.49
Semen pH	6.70±0.23	6.82±0.05	6.73±0.01
Optical Density (O.D.)	0.062±0.014	0.24±0.02	0.26±0.01
Colourimetric sperm concentration ($\times 10^6$/ml)	256.67±74.53	1050.83±106.42	1121.77±36.58
Sperm Concentration/ejaculate ($\times 10^6$)	764.25±309.39	7373.71±998.41	8208.17±500.46
Haemocytometric sperm concentration/ml($\times 10^6$)	47.83±16.49	1152.18±482.81	1235.48±537.79
Live sperm per cent	7.83±3.30	69.63±2.43	89.53±1.30
Dead sperm per cent	92.17±03.20	30.38±2.43	10.98±1.68
Abnormal sperm per cent	41.67±13.73	15.17±1.31	5.76±0.37
Dead sperm % due to cold shock test	100.00±0.00	43.29±3.06	43.51±1.09
Dead sperm % due to hot shock test	94.57±2.18	42.91±2.81	24.76±1.18
Crenellation pattern of semen (1-3 scale)	1.33±0.37	2.08±0.13	2.23±0.09
IV Seminal Biochemical profiles:			
Initial fructose (mg%)	319.45±136.14	466.90±55.01	540.59±19.38
Citric acid (mg%)	350.00±34.22	355.72±25.45	386.73±76.83
Total sialic acid (mg%)	29.39±11.41	50.51±3.46	53.30±1.31
Free sialic acid (mg%)	4.79±1.01	4.28±0.78	3.90±0.24
Bound sialic acid (mg%)	24.60±13.32	46.23±3.39	49.37±1.31
Total protein (gm%)	6.49±0.19	9.56±0.39	10.29±0.13
Total solids (gm%)	4.70±0.10	10.66±0.59	12.51±0.20
Calcium (mg%)	33.94±3.64	40.89±3.23	45.68±0.91
Inorganic phosphorus (mg%)	11.59±5.51	16.69±1.03	14.59±0.45
Ca: P ratio	2.93	2.45	3.17
Magnesium (mg%)	4.83±0.50	4.26±0.18	3.85±0.11
Sodium (Na) mEq/L	151.26±4.75	159.43±5.07	148.12±1.91
Potassium (K) mEq/L	25.02±16.13	34.56±3.24	20.24±5.49
Na : K ratio	6.04	4.59	7.38

Figures in parentheses indicate number of observations.

In case of bull Rosy, mass motility, individual sperm motility and live sperm per cent were significantly lower, while dead and abnormal sperm per cent and dead sperm per cent due to hot shock test were significantly higher than the values observed in 7 normal bulls (Table-1). The sperm abnormalities encountered were of head, mid piece, tail and protoplasmic droplets as 3.25, 5.12, 6.39 and 6.31 per cent, respectively. Among the cytoplasmic droplets, 64.83 and 35.17 per cent were of proximal and distal, respectively. Most of the sperms were narrow at the base with damaged acrosomal cap and detached head. This semen picture denoted severe degenerative changes in the testicles. However, rest of the seminal characters were nearly normal. The gross testicular size and scrotal circumference were in the normal limits. The freezability of semen (post-thaw motility 42.17%) and fertility (of 215 frozen semen insemination, 41.86%) in this bull were significantly low as compared to other normal bulls (62.71% and 47.93%, respectively). All these findings are in close confirmity with those reported by Settergren (1978) and Silva *et al.* (1982) in cases of low germ cell resistance hypoplasia. The later authors have reported a hereditary familial occurrence of LGCRH in Santa Gartrudis bulls with an average CR of sire as 65.67% vs 79.46% for other normal bulls in the area. The sperm motility in sire and his 5 sons ranged from 11.67±7.64 to 75.00±7.07% with the percentage of major and minor sperm defects as 19.09±7.78 to 66.5±7.86% and 12.0±1.41 to 34.2±17.31%, respectively.

Seminal biochemical profiles:

The values of seminal biochemical profiles studied were lower for all constituents except for free sialic acid, sodium, potassium, sodium: potassium ratio and magnesium which were apparently and/or significantly higher in both the affected bulls (Table 1). Doicheva (1981) and Schons *et al.* (1975) observed significantly low levels of seminal fructose and citric acid in bulls with infertility problems like hypoplasia and degeneration. This is in agreement with the present findings. However, Pedroso *et al.* (1979) reported higher contents of seminal plasma calcium and phosphorus in 10 HF bulls with testicular degeneration as compared to normal bulls. These findings to some extent coincided with the levels of inorganic phosphorus obtained in the LGCRH bull Rosy which had subsequent testicular degeneration.

Histo-pathological studies:

Bull Rosy: On histo-pathological studies of testes from bull Rosy, the sections of left testicle showed congestion. Seminiferous tubules showed various grades of degenerative changes and were thinned-out due to considerable decrease in the cellularity. The tubules were lined by spermatological cells few in number with thickening of basement membrane. Some tubules showed vacuolar degeneration of tubular epithelium while other showed necrotic mass in the lumen. In some tubules sperms were observed in the lumen. Further, differentiated stages of cells were hardly seen in most of tubules. Proportionate reduction in leydig cells population was noticeable. Second section of the same testicle showed seminiferous tubules to contain cells in 2 to 3 lines with differentiation, although inadequate in nature. Few tubules indicated degenerated spermatozoal cells.

The sections of the right testicle showed seminiferous tubules with spermatological lining cells in-turn followed by several differentialed cell layers. The cytoplasm

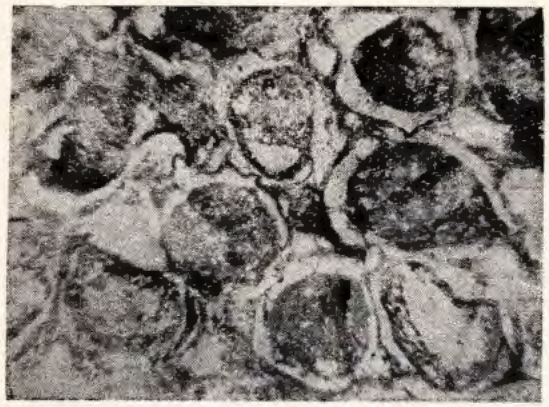

Fig. 2; HP section of testis from LGCRH bull-Rosy (HE × 300): note the necrosed degenerated sperm masses in the lumen of seminiferous lubules

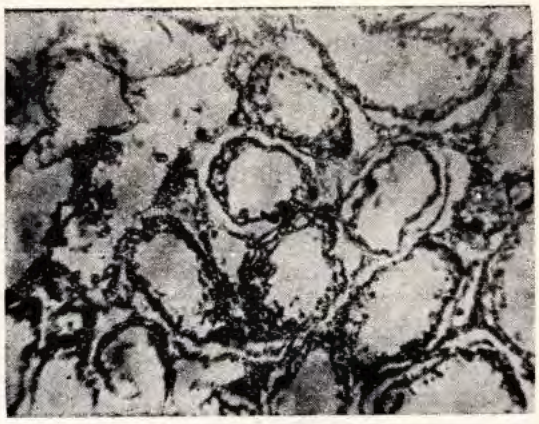

Fig: 3: HP section of testics from Bull Deolo: Bilateral testicular hypoplasia (HE × 300 approximate) note complete arrestment of spermatogenesis process.

of cells was vacuolated and degenerative changes were evident. The cellularity of the tubules was considerably reduced. However, leydig cells appeared to be normal in number and morphology (Fig. 2).

Silva *et al.* (1982) carried out histopathological studies of testes from 3 of the affected sons of a LGCRH Santa Gartrudis bull. The findings revealed alterations in the seminiferous tubules and the presence of giant multinucleated cells in the lumen and vacuolation & pyknosis of primary spermatocytes. They concluded that the bulls had testicular hypoplasia with low germ cell resistance, and that this was probably hereditary in origin. These findings closely agreed with the present findings of LGCRH.

Bull Deolo: The sections of testicles from bull Deolo revealed thickening and hyalinization of the basement membranes of all the seminiferous tubules. Tubules were lined by single or double layers of cells and diameter was smaller than normal. There was complete arrestment of the spermatogenesis process with only few primary spermatocytes and spermatogonia lining the tubules (Fig. 3) as against several stages of spermatogenic cells in normal bulls testes. Interstitial cells were also scanty. The sections of right and left testicles showed similar changes.

Left sided testicular hypoplasia seems to be more common than right sided and/or bilateral cases (Lagerlof, 1938; Rao *et al.*, 1966; Kodagali and Kerur, 1968; Kodagali *et al.*, 1971; Kodagali 1977; Kaikini *et al.*, 1978; Rao, 1978). Narsimhan *et al.* (1981) and Deshpande *et al.* (1976) reported bilateral testicular hypoplasia in cross-bred and HF bulls respectively, which were diagnosed on the basis of semen evaluation and histopathological examination of biopsied testicular material. Bongso *et al.* (1982) observed a case of testicular hypoplasia in a bull with XX/XY chimerism associated with arrested spermatogenesis. Phenotypic examination revealed underdeveloped dorsal neck muscle, a non-pendulous scrotum, testes firm in consistency and scrotal circumference of

27 cms. On rectal palpation small vesicles were present.

Settergren (1978) described the characteristics of testicular hypoplasia in the wider sense as congenital, gonads smaller than normal, no or relatively few germ cells produced and cells of inferior quality. In 'Classical' type, there are no germ cells in part of or the whole gonad(s) and it affects both the sexes equally. The condition is usually unilateral and occurs in 88% of cases in the left testicle, in 4% the right testicle and in 8% it is bilateral. It can be partial or complete. In total bilateral hypoplasia, testicles are very small and no sperms are produced. However, serving ability is not impaired; on the contrary it might be increased. Histologically, seminiferous tubules are devoid of germ cells and only single layer of undifferentiated sertoli cells is present.

Further he explained that in 'LGCRH' type, there are germ cells present in the seminiferous tubules but the number is often low; the development of sperm is abnormal or the resistance of sperm to freezing or storage is low; sometimes fertility is low inspite of normal looking semen; the testicles can vary in size from very small to normal and it seems to be more common in males. This type of hypoplasia first occurred in sons of a particular sire which had fertility 2-5% below the normal average. Out of 45 sons tested, 50% were rejected because of poor semen or low fertility. He stated that the history and clinical signs may vary to a large extent. Heavily affected bulls may show aspermia and very small testicles since young age. Others may have normal semen and fertility when they are 12-14 months old but their fertile life is very short and at the age of 18-20 months they started to show signs of testicular degeneration and 3-6 months later, they could no longer be used. The diagnosis must therefore be based on both history and repeated clinical examinations over 6-12 months. In SRB bulls the defect had occurred in certain lines within the breed. The semen picture had different degree of testicular degeneration. On histological examination, all the seminiferous tubules had at least some germ cells even if the numbers were very low and degenerated, in contrary to 'Classical' type of hypoplasia.

The third type of hypoplasia which occurred in Swedish Friesian Breed (SLB) and characterised by no transformation of spermatids to spermatozoa, was seen only in males and was always bilateral. The testicles were of almost normal in size and consistency but semen picture showed aspermia or very low sperm not exceeding 200×10^6/ml with all sperms morphologically abnormal (Settergren, 1978).

Barba and Montes (1982) studied the main testicular changes in 44 infertile cross-bred bulls on slaughter. Of these 4, 22, 12, 1 and 2 bulls had slight, moderate & severe testicular degeneration, testicular hypoplasia and orchitis, respectively. The findings reported by them agreed with the present findings.

Considering the testicular measurements, semen characters, seminal biochemical profiles, fertility results and testicular histopathological studies, the bull Rosy was diagnosed to have low germ cell resistance hypoplasia and bull Deolo had complete bilateral testicular hypoplasia.

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Effect Of Exogenous And Endogenous Oxytocin On The Conception Rate In Cross-bred Cattle

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ABSTRACT

The study was conducted on 104 cross-bred cattle. The conception rate was found to be much higher in all the treatment groups as compared to the control. Highest conception rate (83.33%) was observed in cows when 50 I.U. of Oxytocin was administered intramuscularly, five minutes before insemination. It was followed by 80% conception rate in cows when udder masage was done for two minutes, five minutes before insemination. In the case of heifers, a conception rate of 83.33%, which was the maximum when compared to all other treatments, was obtained where clitoral stimulation was done for 10 seconds, 5 minutes before insemination.

* * *

Numerous factors have been reported for lowered conception rate in cattle. Nervousness and excitement at the time of insemination has been considered to be one of such factors—the low conception rate resulting due to defective sperm transport (Vandemark and Hays 1953, 1954). It has been further reported that Oxytocin liberated by stimulation of external genitalia at coitus induced uterine contractions and helped in sperm transport (Harris, 1947). Present study was therefore, conducted to investigate the effect of exogenous Oxytocin administration and endogenous release of Oxytocin by clitoral stimulation and udder massage on conception rate in cross-bred cattle.

Materials and Methods

The study was conducted on 104 cross-bred cattle (72 cows and 32 heifers) brought to the Department of Gynaecology, Ranchi Veterinary College for artificial insemination. Only those cows and heifers were included in the trial which had apparently normal genitalia and were free from infection. Cows included in the study had calved recently and were showing either first or 2nd post-partum oestrus. Artificial insemination was done during mid-heat with semen obtained from pure Holstein Friesian bulls which was diluted in E Y C extender.

The experimental animals were randomly divided into three groups. Group I-consisting of 25 animals (18 cows and 7 heifers)-was kept as control. In this group, only insemination was done and no treatment was given. Animals under group II were further categorised under two subgroups (a & b). Group II (a) comprised of 18 animals (13 cows and 5 heifers) and group II (b) consisted of 20 animals (12 cows and 8 heifers). The animals under group II (a) received 30 I.U. of Oxytocin and those of group II (b) received 50 I.U. of Oxytocin intramu-

TABLE 1. Effect of different treatments on conception rate.

Experimental Treatments		No of animals inseminated			No. of animals conceived		
		Cows	Heifer	Combined (Cow+Heifer)	Cow	Heifer	(Cow+Heifer)Combined
I.	Control (No treatment)	18	7	25	8 (44.44)	3 (42.86)	11 (44.00)
II.(a)	Oxytocin 30 I.U.	13	5	18	8 (61.54)	3 (60.00)	11 (61.11)
II.(b)	Oxytocin 50 I.U.	12	8	20	10 (83.33)	5 (62.50)	15 (75.00)
III.(a)	Clitoral stimulation	14	6	20	11 (78.57)	5 (83.33)	16 (80.00)
III.(b)	Udder massage	15	6	21	12 (80.00)	3 (50.00)	15 (71.43)

Figures in parentheses denote percentage.

TABLE 2. Normal deviate test values between different groups in Cows.

Experimental group	Oxytocin group		Stimulation group	
	30 I.U.	50 I.U.	Clitoral stimulation	Udder massage
Control	0.942	2.132*	1.950	2.08*
Oxytocin 30 I.U.	—	1.212	0.968	1.080
Oxytocin 50 I.U.	—	—	0.235	0.210
Clitoral stimulation	—	—	—	0.094

* = Significant at 5% level

scularly five minutes before insemination. Animals under group III were also categorised into two subgroups (a & b). Group III (a) comprised of 20 animals (14 cows and 6 heifers) and each animal of this group was given clitoral stimulation for 10 seconds five minutes before insemination. The animals of group III (b) consisting of 15 cows and 6 heifers were subjected to udder massage for two minutes, five minutes before insemination. For this purpose a clean piece of cloth was soaked in luke warm water, squeezed and used for sponging of the udder for two minutes.

Pregnancy was confirmed by rectal palpation, 45-60 days after insemination. Statistical analysis was done according to the methods suggested by Snedecor and Cochran (1968).

Results and Discussion

Oxytocin and conception rate:

Table 1 shows the conception rate in different experimental groups. It is evident from the table that in all the treatment groups the conception rates were much higher than that of control. The conception rate in cows, heifers and the combined category was 61.54, 60.00 and 61.11 percent respectively when 30 I.U. of Oxytocin was administered, whereas the conception rates were 83.33, 62.50 and 75.00 percent in the same sequence when 50 I.U. of Oxytocin was given intramuscularly five minutes before insemination. The difference in the conception rates between the control group and group II (b) was significant ($p<0.05$), while it was non-significant

TABLE 3. Normal deviate test values between groups in heifers.

Experimental group	Oxytocin group		Stimulation group	
	30 I.U.	50 I.U.	Clitoral stimulation	Udder massage
Control	0.585	0.760	1.495	0.257
Oxytocin 30 I.U.	—	0.090	0.865	0.331
Oxytocin 50 I.U.	—	—	0.853	0.467
Clitoral stimulation	—	—	—	1.224

TABLE 4. Normal deviate test values between different groups in combined unit (Cows+Heifers)

Experimental groups	Oxytocin group		Stimulation group	
	30 I.U.	50 I.U.	Clitoral stimulation	Udder massage
Control	1.107	2.092*	2.449*	1.869
Oxytocin 30 I.U.	—	0.919	1.282	0.681
Oxytocin 50 I.U.	—	—	0.378	0.257
Clitoral stimulation	—	—	—	0.638

* = Significant at 5% level

between the control and group II (a) (Table 2 & 4). However, the difference was not significant in respect of heifers (Table—3). In the present study it was observed that the conception rate was highest (83.33%) when 50 I.U. of Oxytocin was administered which is in agreement with the findings of Daniel and Venkatasami (1972) who also observed better conception rate with 50 I.U. of Oxytocin. The present finding is quite contrary to the results of Singh and Gangwar (1976) who found an increase in conception rate by 13.6% against control when they injected 15 I.U. of Oxytocin 5 minutes before insemination. They could not get any advantage by raising the dose of Oxytocin to 30 I.U. while a dose of 45 I.U. was found to be less effective.

The animals in heat are nervous and excited and are put to stress while artificial insemination is done. This leads to liberation of epinephrine i.e. fright hormone. In cows, epinephrine causes a relaxation of the oestrogenised uterine muscle thus affecting sperm transport and fertilization rate (Hays and Vandemark, 1952; Vandemark and Hays, 1953). Hays *et al.* (1958) observed that a deficiency in the release of Oxytocin occurred in some cows which affected the conception rate. They further observed that the conception rate could be improved by administration of Oxytocin at the time of breeding.

In the present study, the exogenous Oxytocin administered five minutes before insemination is thought to have neutralized the epinephrine which was released due to nervousness and thus improved the conception rate.

Clitoral stimulation and conception rate:

The conception rates were 78.57, 83.33 and 80 per cent in cows, heifers and the combined group respectively when clitoral stimulation was done for 10 seconds, five minutes before insemination (Table 1). The difference in the conception rates between control and group III (a) was statistically significant (Table-4). The present finding was in agreement with the results of Anon (1975)

and Randel *et al.* (1975) who reported an increase in conception rate by clitoral stimulation. However, Thompson *et al.* (1975) and Refsdal (1978) could not observe any significant rise in conception rate by clitoral stimulation. The clitoris which is homologous to penis is a rudimentary structure in females. It is highly vascular and innervated. Its massage or touch caused sufficient sex stimulus and impulses are carried to the hypothalamus due to which Oxytocin is released from the posterior pituitary (Roberts, 1971; Thompson *et al.*, 1975). It is due to these properties of clitoris that its stimulation led to high conception rates during the present study.

Udder massage and conception rate:

The conception rates in cows, heifers and combined group treated under group III (b) were found to be 80.00, 50.00 and 71.43 per cent respectively. The only available reference on this type of study was that of Ivanov (1967) who reported that udder massage led to increased conception rate in cows. Earlier workers like Denamur (1965), Barowick and Ewy (1973) Sibaja and Schmidst (1975) and Worstorff *et al.* (1980) reported the release of Oxytocin by the posterior pituitary as a result of mammary massage. The Oxytocin liberated due to udder massage increased the motility of the uterus which was pre-sensitized with oestrogen.

This led to rapid transport of sperms to the oviducts and increased conception rate as observed during the present study. In the case of heifers, udder massage did not bring about marked improvement in conception rate which might be due to the fact that udder in heifers is not developed and its surface area also is not sufficient due to which effective massage is not possible. In heifers probably less Oxytocin was liberated due to which conception rate did not increase markedly. The higher conception rate observed during the present study either due to the exogenous Oxytocin injected before artificial insemination or endogenous Oxytocin released due to clitoral or udder stimulation was obtained probably because Oxytocin neutralized the effects of epinephrine which resulted in higher conception rate.

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SHORT COMMUNICATIONS

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Prediction Of Post-Insemination Reproductive Status In Buffaloes

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ABSTRACT

One hundred and nineteen Surti heifers/buffaloes exhibiting first post-partum oestrus were inseminated once in the month of March and were subsequently followed by rectal palpation and vaginal inspection on 18-24 days and 60 days post-insemination for prediction and confirmation of future reproductive status. This was based on palpable changes in the ovarian structures and the uterus and visible signs. The predictions that the animals would be pregnant, would evince oestrus or enter into anoestrum based on 18-24 days examination were 42.02 %(50), 38.66 %(46) and 19.33 %(23) respectively. The accuracy of the prediction for pregnancy, oestrus/cyclicity and anoestrum was 68.00 % (34), 95.65 % (44) and 100.00 % (23) respectively, based on confirmatory findings made around 60 days post-insemination. The overall first inseminations conception rate obtained with liquid semen in the month of March was 28.57 % (34/119) requiring on an average 3.50 inseminations per conception. The study proved the practical applicability and usefulness of rectal palpations on 18-24 days post-insemination/service and observance of the visible signs for predicting probable future reproductive performance in buffaloes under field conditions.

* * *

The clinician's ability to observe, palpate and diagnose clinically detectable changes in the ovarian structures permiting the determination of ovarian activity and prediction of future reproductive status in bovines is extremely important under field conditions. Where there is a limitation of the usage of highly sophisticated technique like RIA for plasma/milk progesterone assay for prediction of early pregnancy and the cost involved.

In buffaloes, reports on ovarian palpable changes and the observance of visible signs to predict future reproductive status post-insemination/oestrus are apparently not available. Therefore, a study was undertaken in Surti buffaloes post-insemination by examining the visible signs and the palpable changes in the ovarian structures and uterus per-rectally.

Materials and Methods

The study was conducted on 23 heifers and 96 first post-partum oestrus buffaloes (119) of Surti breed, at the Gujarat Veterinary College AI Clinic, Anand during a period of 3 months from March to May, 1984. All these animals were in normal body condition and were presented at the clinic by individual owners for inseminations and subsequent follow-up. Before insemination, the detailed history, oestrus signs and the findings of vaginal inspection and rectal exploration

TABLE 1. Prediction of reproductive status post-insemination by rectal palpation findings and vaginal inspections in Surti buffaloes.

Sr. No.	Particulars	Heifers	Buffaloes	Total
1.	No. of animals inseminated	23	96	119
2.	Prediction on 18-24 days post-insemination for			
	a) Pregnancy.....	8 (34.78%)	42 (43.75%)	50 (42.02%)
	b) Oestrus.....	9 (39.13%)	37 (38.54%)	46 (38.66%)
	c) Anoestrum.....	6 (26.09%)	17 (17.71%)	23 (19.33%)
3.	Confirmation on 60 days post-insemination and the accuracy of prediction (%)			
	a) found pregnant..	6 (75.00%)	28 (66.67%)	34 (68.00%)
	b) evinced oestrus.	7 (77.78%)	37 (100.00%)	44 (95.65%)
	c) entered anoestrus	6 (100.00%)	17 (100.00%)	23 (100.00%)
4.	Animals conceiving to first/fresh AI and CR %	6 (26.09%)	28 (29.17%)	34 (28.57%)
5.	No. of inseminations per conception	3.83	3.43	3.50

were recorded. Animals were inseminated at their peak of oestrus by recto-vaginal technique using freshly collected semen extended in citrate diluent. The owners were asked to watch their animals for recurrence of oestrus signs, if any and bring them between 18-24 days post-insemination and subsequently around 60 days for re-examination. The criteria used on 18-24 days post-insemination examination for probable predictions of early pregnancy, recurrence of oestrus or entering into anoestrum were: presence or absence of CL and graafian follicle, their size, nature, consistency, shape as well as ovarian size and tonicity or flacidity of uterine horns etc. Those animals which repeated oestrus were re-bred by AI. All the animals were re-examined 3rd time around 60 days of first AI for confirmative diagnosis of probable predictions made on 18-24 days post-insemination examination. The accuracy of predictions for pregnancy, oestrus and anoestrus was worked out based on findings of fully developed CL and thinning & asymetry of gravid horn, foetal membrane slip and palpation of amniotic vesicle for pregnancy; presence or development of graafian follicle or palpation of CL with crown for recurrence of oestrus and smooth, round, small, inactive ovaries devoid of CL or follicular development for anoestrum.

Results and Discussion

In all, 23 heifers and 96 Surti buffaloes with first post-partum oestrus was artificially inseminated after detailed gynaeco-clinical examination. These were subsequently followed either at the clinic or at the owner's door between 18-24 and around 60 days post-insemination for prediction and confirmation of pregnancy, evincing of oestrus or entering into anoestrum. The details of the findings about predictions and confirmations have been presented in Table-1.

It can be seen from the table that on 18-24 days examination, out of total 119 heifers and buffaloes inseminated, 50 (42.02%), 46 (38.66%) and 23 (19.33%) animals were predicted for probable pregnancy, recurrence of oestrus and entering into anoestrum, respectively. Of these, 34 animals were actually found pregnant, 44 were in oestrus/cyclicity i.e. had passed the oestrus and 23 were found to be anoestrus, when confirmed around 60 days post-insemination. The accuracy of predictions made for pregnancy, oestrus/cyclicity and anoestrum on 18-24 days examination was 68.00, 95.65 and 100.00%, respectively. The accuracy of prediction for animals turning anoestrus was cent per cent in both heifers and buffaloes. While, accuracy for prediction of pregnancy was 75.00 and 66.67% and for oestrus 77.78 and 100% in heifers and buffaloes, respectively.

Dawson (1975) reported on the correctness of ovarian structures palpated per-rectally in 83% cases based on 85 cows slaughtered 24 hrs after the examination. Clinically, the CL was diagnosed accurately in 85% ovaries. Koeshy (1983) examined 240 HF × Hungarian Pied cows twice between days 8-14 and 18-24 after AI and the accuracy of correct diagnosis for pregnancy was reported to be 72.06%, which is in agreement with the present findings of 68.00% in buffaloes.

The overall number of buffaloes and heifers conceiving to first inseminations performed in month of March were 34 (28.57% CR) requiring 3.50 inseminations per conception. Dhami and Kodagali (1986) reported first service conceptions for frozen semen inseminations performed in the month of March to be 26.37% in Surti buffaloes.

The results indicated that the gynaeco-clinical examination of animals inseminated before 18-24 days can serve useful purpose to anticipate future reproductive status in bovines.

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Ovulatory Disturbances In Repeat Breeding Cross-bred Cattle

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ABSTRACT

A total of 97 oestrous cycles in 28 repeat breeding cows and 9 heifers were studied clinically in respect of ovulatory disturbances. The extent of delayed and anovulatory conditions in heifers were found to be 29.41 and 35.29 per cent and 52.50 and 22.50 per cent in cows, respectively. A total of 24 repeat breeding crossbred cows and heifers were treated with progesterone, triple sulphate mixture and chorionic gonadotrophin resulting into 33.33, 66.66 and 83.33 per cent pregnancies, respectively.

* * *

Detailed clinical examination of the reproductive organs is possible in bovines. This provides valuable information about the reproductive phase in the individual animal. With the emphasis on breeding efficiency, especially in dairy herds routine rectal explorations are frequently performed for sexual health control. Clinical changes in the ovary are dependent upon the content i.e. whether follicles or corpora lutea. The literature regarding ovulatory disorders in crossbreds is scarce and hence this study was undertaken and the findings have been reported.

Materials and Methods

A total of 97 oestrous cycles in 37 (28 cows and 9 heifers) repeat breeding crossbred animals belonging to Livestock Research Station, Anand were clinically investigated. The criteria for delayed ovulation and anovulation pattern were according to Kavani (1984). These animals were treated with 3 treatment regimes—keeping six repeat breeding animals under control group. Treatments included (i) Prolution depot (25 mg) given by deep I/M injection as soon as the animal was noted in oestrus, (ii) Triple sulfate mixture ($CuSO_4$ 150 mg; $CoSO_4$ 2 mg and $FeSO_4$ 1000 mg) was given daily for ten days beginning with the day of oestrus and (iii) Chorionic gonadotrophin B.Vet. C. luteinising hormone, 1500 IU (Chorulon, Intercare) was given intravenously during oestrus followed by insemination.

Results and Discussion

The observations made and the results of treatment in delayed and anovulatory conditions have been presented in Table 1 and 2.

From the Table 1 it can be observed that delayed ovulation condition was more than the anovulation (48.45 Vs 24.74 per cent). Also, delayed ovulation was more in cows than in heifers (52.50 Vs 29.41 per cent). However, anovulation was more in heifers than in cows (35.29 Vs 22.50 per cent). Van Rensburg and Devos (1962) reported delayed ovulation

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TABLE 1. Ovulatory distrubances in repeating crossbreds.

Crossbreds	No. of animals	No. of oestrous cycles	Delayed ovulation %	Anovulation %
Heifers	9	17	29.41 (5)	35.29 (6)
Cows	28	80	52.50 (42)	22.50 (18)
Total	37	97	48.45 (47)	24.74 (24)

Figures in parentheses indicate number of oestrous cycles

TABLE 2. Results of treatment in ovulatory disturbances

Treatment	No. of animals	Conception rate %	Inter-oestrual length (days) Pre-treatment	Post-treatment
Progesterone	9	33.33	34.30±2.29	20.37±0.98
Triple Sulph	9	66.66	32.65±2.52	25.25±2.48
Chorionic gonadotrophin	6	83.33	27.08±1.51	21.22±1.28
Overall treated group	24	58.33	31.34±1.78	21.67±1.46
Control	6	16.16	32.35±2.32	32.35±2.32

to the extent of 66.0 per cent. Hancock (1948) and Maree (1977) reported lower incidence of 31.0 and 17-34 per cent, respectively. The incidence observed in the present study is intermediate between these values.

The overall incidence of anovulation observed in the present study was 24.74 per cent. This was higher than the incidences viz., 18.0, 17.4, 5.0, 17.66 and 9.4 per cent reported by Van Rensburg (1956), Glod (1961), Morrow (1969), Maree (1977) and Mathai and Raja (1978), respectively.

Out of 24 repeat breeding animals treated 58.33 per cent animals became pregnant as compared to 16.66 per cent in the control group. The animals conceiving in Progesterone treatment group, Triple sulph group and in Chorionic gonado-trophin group were 33.33 66.66 and 83.33 per cent, respectively. Overall results of pregnancy in treated groups were found to be significantly higher ($P<0.05$) than the control group. The effect of treatment on the inter-oestrual length is evident from Table 2. It was 31.34±1.78 days pretreatment which became 21.67±1.46 days post-treatment. Perhaps this might be the reason for the conceptions in the repeating animals. The correction in the oestrous cycle rythum might have corrected the ovulatory disorders in progesterone and triple sulphate treated animals. The percentage of pregnancies obtained (83.33 per cent) with chorionic gonadotrophin is high on account of drug being specific for ovulatory disorders.

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Ejaculate Characteristics Of Saanen, Barbari And Cross-bred Bucks

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Ejaculate characteristics of goats are influenced by breed (Orson and Victor, 1952; Eaton and Simmons, 1952 and Kurian and Raja, 1965). Very few reports are available on this trait of Saanen, Barbari and their cross-bred bucks (Tiwari *et al.*, 1968; Sahni and Roy, 1969 and Koh, 1975). The present study was therefore, conducted to characterise and compare the semen traits of the three breeds of goats.

Materials and Methods

Twelve healthy breeding bucks (four each of Saanen, Barbari and Saanen × Barbari crosses) of approximately 2-3 years of age and reared nnder similar managemental conditions were selected for this study. Semen was collected from individual buck at weekly interval in artificial vagina. Immediately after collection semen samples were assessed for different seminal traits. Ejaculate volume was read directly from the graduated collecting tube. The sperm concentration was determined by standard haemocytometer method, the live-dead count and morphological examinations using eosin-nigrosin stain (Hancock, 1951) and pH by digital pH meter. Both the inter-and intrabreed results of the spermiogram were compared statistically according to Snedecor and Cochran (1968).

Results and Discussion

The mean values of different semen traits for all the three breeds of bucks are shown in Table 1. Both the quality and quantity of the semen were comparable in all the three breeds, and that all the mean values were within the range reported by different authors (Tiwari *et al.*, 1968; Sahni and Roy, 1969 and Singh *et al.*, 1982). The critical difference test revealed that, there were significant interbreed differences in mean concentration of sperm cells ($P<0.01$), the mean percentage of total morphological sperm cell abnormalities ($P<0.01$) and pH of semen ($P<0.05$), while the interbreed differences in ejaculate volume, mass-motility and percent live cells were not significant.

Semen characteristics vary with factors such as age, body weight, season, temperature, methods, time and frequencies of collection, level of nutrition and physiological conditions of the animal (Zemjanis, 1970; Morrow, 1980 and Ott and Memon, 1980). Most of these factors being common in all the breeds studied. The variations in some of the semen traits in the present study could be partly attributed to breed and body weight of the animals.

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TABLE 1: Mean values of different semen traits of Saanen, Barbari and cross -bred bucks.

Breed	No. of bucks	No. of ejaculates	SPERMIOGRAM					
			Volume (ml)	Mass activity (0—5)	Concentration (million/ml)	Live cells (%)	Total abnormal cells (%)	pH
Saanen	4	52	0.70±0.022	4.16±0.075	2820.96±7.427^a	91.13±0.986	7.54±0.449^a	6.60±0.002^a
Barbari	4	51	0.72±0.032	4.35±0.06	2117.65±32.445^b	92.53±0.291	5.94±0.252^b	6.78±0.002^b
S×B cross	4	53	0.69±0.024	4.18±0.068	2375.47±7.095^c	91.07±0.544	7.63±0.442^c	6.68±0.002^c

Values with different superscript in a column differ significantly

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Effect Of Thawing Methods On Quality Of Frozen Goat Semen

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Studies on effect of thawing methods on quality of frozen goat semen are very scanty. Rossouw (1974) and Waide *et al.* (1977) studied sperm motility in relation to thawing temperature. The present investigation was undertaken to record the effect of rapid and slow thawing methods on sperm motility, livability and acrosomal morphology of buck spermatozoa.

Materials and Methods

One hundred and seventy two ejaculates obtained from 5 native goats (*Capra hircus*) were frozen in 0.5 ml French straws by exposing for 10 minutes to liquid nitrogen vapour, 5 cm above the liquid nitrogen level and then stored in liquid nitrogen. Thawing of frozen semen was done by immersing the straws in water at 37°C for 12-15 seconds (rapid) or at 5°C for 2 minutes (slow). After thawing, the semen of two straws from the same ejaculate and thawed by same method was pooled together and evaluated for percentage of progressively motile sperm, live sperm and damaged acrosomes. The percentage of progressively motile sperm was estimated at a magnification of 450× using a phase contrast microscope. The percentage of live sperm was determined using eosin-nigrosin stain (Blom, 1977). The incidence of damaged acrosomes was studied using Giemsa staining technique of Watson (1975). Two hundred sperms were counted in each smear at a magnification of 1000× and different forms of damaged acrosomes were classified as per Watson and Martin (1972). The statistical analysis of the data was done as per Snedecor and Cochran (1968).

Results and Discussion

The results of this study are presented in Table 1. The significantly higher ($P<0.01$) percentages of progressively motile and live sperms recorded in this study after rapid thawing than slow thawing support the observations of other workers on ram and bovine semen (Blackshaw, 1955; Rodriguez *el al.*, 1975; Munoz, 1979; Vera, 1980 and Pace *et al.*, 1981). The different forms of damaged acrosomes did not differ significantly between thawing methods. This is in agreement with that of Olar *et al.* (1977). In contrary, other workers recorded significantly higher percentage of intact acrosomes when bovine semen was thawed at 37°—40°C than at 5°C (Munoz, 1979; Vera, 1980 and Pace *et al.*, 1981). The significantly higher ($P<0.01$) percentage of progressively motile and live sperm and slightly less acrosomal damage observed after rapid thawing than slow thawing might be due to short period of exposure of sperm to increased solute concentrations during rapid thawing.

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TABLE 1. Percentage of progressively motile sperm, live sperm and various forms of damaged acrosomes (Mean ± SE) in frozen goat semen after thawing by two methods.

Thawing methods	Progressively motile sperm% (172)	Live sperm% (172)	Swollen acrosome % (134)	Separating acrosome% (134)	Entirely lost acrosome% (134)	Total damaged acrosomes% (134)
Rapid thawing (37°C for 12-15 sec.)	62.89±1.06	65.63±0.98	13.02±0.77	0.74±0.10	1.78±0.09	15.50±0.81
Slow thawing (5°C for 2 min.)	53.28±1.15	55.47±1.11	14.54±1.04	0.95±0.11	1.64±0.11	17.05±1.07
't' value	6.16**	6.85**	0.73NS	1.48NS	1.57NS	0.87NS

Figures in parentheses indicate number of observations.

** = $P < 0.01$, NS = Non-significant

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Preservation Of Boar Semen

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ABSTRACT

Split samples from each of ten ejaculates collected at three days interval from four Landrace boars, were diluted in Glucose Potassium sodium tartrate-Sodium citrate dihydrate Edate (GPSE-I) Glucose-Sodium citrate dihydrate Edate (GPSE-II), Glucose Potassium sodium tortrate Sodium citrate dihydrate (GPSE-III), Egg yolk Glucose Sodium bicarbonate (EYGS) and BL-1 diluents. Preservability of boar spermatozoa were recorded from 0 hour upto 96 hours of preservation at 24 hours interval and the mean preservability was recorded to be 84.65, 77.55, 71.80, 63.50 and 50.45 per cent respectively in GPSE-I diluent; 80.75, 71.22, 64.35, 54.52 and 40.35 per cent respectively in GPSE-II diluent; 82.15, 64.75, 48.62, 34.02 and 16.87 per cent respectively in GPSE-III diluent; 74.10, 66.17, 57.17, 46.37 and 26.95 per cent respectively in EYGS diluent and 76.72, 67.92, 50.17, 33.50 and 16.75 per cent respectively in BL-1 diluent. The variation of preservability of boar spermatozoa between diluents and between hours of preservation was found to be significant ($P<0.01$).

* * *

The magnitude of semen preservation is based on the preservability of spermatozoa in the diluents and the aftermath fertility results. It is imperative to undertake sufficient trials on preservation of boar semen before adopting artificial insemination in pigs. In India, except two isolated reports (Radhakrishna Murty, 1974 and Iyer, 1978), no further study has been carried out till to-day. Here, in this endeavour a preliminary comparison has been made with three new diluents, GPSE-I, GPSE-II and GPSE-III (Tamuli, 1982).

Materials and Methods

Ten semen ejaculates from each of four Landrace boars (aged 15 to 18 months) were collected at three days interval in the thermosflask through a nylon strainer over the dummy sow. The gel masses and the pre-sperm fractions were eliminated from the ejaculates. Split samples from each ejaculate were diluted at the ratio of 1:3 in the diluents, GPSE-I (Glucose Potassium sodium tartrate Sodium citrate dihydrate Edate), GPSE-II (Glucose Sodium citrate dihydrate Edate), GPSE-III (Glucose Potassium sodium tartrate Sodium citrate dihydrate), EYGS (Egg yolk Glucose Sodium bicarbonate; Dzuik, 1958) and BL-1 (Pursel *et al.*, 1973). The compositions are as follows:

All the diluents were incorporated with Penicillin 60.0 mg, Streptomycin 100.0 mg and Gentamycin 3.2 mg in each 100 ml of diluent and after dilution, preserved at 15°C in neutral glass tubes of 30 ml capacity. The percent motility

Ingredients	GPSE—I	GPSE—II	GPSE—III	EYGS	BL—1
Glucose, gm.	3.5	3.5	3.5	3.0	2.9
Potassium sodium tartrate, gm. :	1.0	—	1.0	—	—
Potassium chloride, gm.:	—	—	—	—	0.03
Sodium citrate dihydrate, gm.:	0.3	0.3	0.3	—	1.0
Sodium salt of EDTA, gm.	0.2	0.2	—	—	—
Sodium bicarbonate gm.	—	—	—	0.15	0.2
Egg yolk, ml.	—	—	—	30	—
Distilled water, ml.	100	100	100	upto 100	100

TABLE 1. Preservability of Boar Semen at Different Hours of Preservation (in percentage).

Hours of preservation	GPSE-I Mean±SE	GPSE-II Mean±SE	GPSE-III Mean±SE	EYGS Mean±SE	BL-1 Mean±SE
0 hour	84.65±0.55	80.75±0.47	82.15±0.45	74.10±0.47	76.72±0.56
24 hours	77.55±0.49	71.22±0.60	64.75±0.71	66.17±0.61	67.92±0.52
48 hours	71.80±0.73	64.35±0.83	48.62±1.20	57.17±0.95	50.17±0.15
72 hours	63.50±1.10	54.52±1.19	34.02±1.51	46.37±1.31	33.50±0.82
96 hours	50.45±1.55	40.35±1.47	16.87±1.67	26.95±2.11	16.75±0.88

was recorded on visual appraisal just after dilution at 0 hour and at 24 hours interval upto 96 hours of preservation. The data were subjected to Arcsin transformation and appropriate statistical analyses were adopted (Steel and Torrie, 1980) for interpretation of the results.

Results and Discussion

The results of mean percent motility at different hours of preservation are presented in Table-1. The preservation of semen varied significantly ($P<0.01$) between diluents and between hours of preservation. The preservability of boar semen was maintained highest in diluent GPSE-I upto 96 hours of preservation where minimum 50 per cent livability was restored. The decreased per cent motility due to preservation in GPSE-II and GPSE-III diluents might be due to the exclusion of Potassium sodium tartrate and Edate, respectively. The BL-1 and GPSE-III diluent maintained a very poor preservability of spermatozoa and they did not differ significantly from each other in respect of their preservable quality (Table-2). Although the BL-1 diluent failed to maintain to the optimum

TABLE 2. Duncan's Multiple Range Test for the Preservability of Boar Semen at Different Hours in Different Diluters.

Diluents		Hours of prservation	
GPSE-I	70.2^a	0 hour	79.9^a
GPSE-II	62.8^b	24 hours	69.7^b
GPSE-III	48.9^c	48 hours	60.3^c
EYGS	54.1^d	72 hours	48.06^d
BL-1	48.7^e	96 hours	29.1^e

Mean values bearing different superscript varied significantly ($P < 0.05$) from each other.

preservability during preservation as mentioned by Pursel *et al.* (1974), but corroberated the findings of Paquignon *et al.* (1979). It was opined that the decreased motility at different preservational hours might be due to the fact that incubation for 30 minutes at 37°C was not carried out before estimation of motility (Pursel, 1982). The per cent motility in EYGS diluent was in close consistence with the findings of Radhakrishna Murty (1974) upto 72 hours of preservation. The significant statistical interaction ($P<0.01$) revealed that the GPSE-I diluent was able to maintain its superiority throughout the periods of preservation.

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Parturition Behaviour In Pigs

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It is the nature which gives shelter to all sorts of living beings and thus the behaviours vary among them in reproduction too. Parturition behaviours have been observed by naturalist (Fraser, 1974 and Signoret *et al.*, 1978) in *sus serofa*,, but reports are not available yet in *Sus vittatus* variety, which are semi-wild in nature. Therefore, separate studies were made of this variety of pigs ('Dom' pigs) in open range as well as in captive condition.

Altogether fifteen pregnant gilts were reared in a privately owned farm in open range condition in one hilly area covered with jungle and *dens* in the neighbourhood of College Campus and in contrast twenty gilts of the same variety were taken in full captive condition managed under A.I.C.R.P. on Pigs in the College. These two groups of pigs were very carefully observed for their pecularity in farrowing behaviours.

In the first group, the pigs used to disappear in the herd all of a sudden before 2 days of farrowing. This missing from the herd usually synchronizes with the swelling of the valva and oedena formation. They usually prefer deep jungle covered with shurbs or on safe den for farrowing. Before farrowing, they prepare their bed in the den or the jungle with dried grass and weak steam of the shrubs. The nest building behaviour has also been observed by Fraser (1974) and Signoret *et al.* (1975). They do not care for food during this period of missing and use to keep themselves aloof for about 4 days. Therefore it was very difficult to say, when actually the farrowing had accurred. The missing pigs could only be identified and the place of farrowing could only be located when they again came for food in the herd after 4 days of missing. When an intruder approaches the den or the nest of farrowing, the pigs produce grunting sounds with aggressiveness to protect their litter (Fig. 1). It is very fantastic to note that some of the pigs ran away from the herd to the place of farrowing bed without finishing the food even, probably

Fig. 1: Parturition behaviour in pigs.
The sow which has farrowed in a close den showed her aggressiveness when the intruder approached.

when they remember that their litter would be in danger in the nest. This symbolizes good mothering behaviour of this variety of pigs. The litter is normally brought to the herd after 6 or 7 days of missing of pigs before farrowing, but never live together in the herd till 13 to 15 days of farrowing.

In captive condition, the behaviour of nest building could not be observed. They remain excited to go out of the pen and peeping through the doors with frequent fickled movement just before two or one day of farrowing. This fickleness in behaviour to go out of the pen gradually decreased in the subsequent second and third farrowings. On the day of farrowing, they become restless for about 3 to 6 hours before the first piglet normally delivered. The farrowing normally occurs early at dawn or in the early morning and is completed within 75 minutes to 190 minutes and the dam takes 5 to 20 minutes to deliver each piglet. Similar observations were also made by Fraser (1974). The placenta weigh 400-1200 gms and normally expelled out within 45 to 105 minutes. The gestation length varies from 106 to 119 days.

The litter size was observed to 3-9 in captive condition, whereas in open range condition, it was only 2-6. The discripancy in the litter size in captive condition might be due to the planned services to the gilts or the death of piglets due to mother's savage is high in open range condition that is due to wildness in behaviour, the dam often use to eat up her neonates after birth. In a separate study of 107 litters, the death of piglets due to mother's savage after farrowing was only 0.6(3) per cent and due to crushing was only 1.4 (7) per cent out of 500 piglets. This indicates good mothering behaviour in comparison to the other breeds of pig where percentage of death due to crushing is high (Smith and Penny, 1981).

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Effect Of Parity On Economic Traits Of Gir Cattle

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ABSTRACT

Breeding phenomenon in Gir cows was studied in order to elucidate the effect of parity on different reproductive and productive parameters. Parity was found to have significant effect on service period, intercalving period and length of lactation, whereas it had no significant effect on weight of male or female calves born or on the gestation period. The study involved an analysis of 285-622 (number) observations for different economic characters.

* * *

For formulating breeding programmes for genetic improvement of dairy cattle and buffaloes on scientific lines, it is necessary to have the basic information on reproductive and produtive characters. Gir being one of the important breeds of dairy cattle in Gujarat State the present study was undertaken.

Materials and Methods

The data pertaining to this study were collected from Virani Goshala, Mangrol, District—Junagadh for the period 1949-72. The goshala was under sexual health control coverage. Age at fertile service, age at calving, weight of male and female calves, service period, intercalving period, gestation length, duration of lactation and milk yield were studied for observations varying from 285 to 622 distributed over twelve lactations. Effect of parity was analysed through analysis of variance as per Snedecor and Cochran (1967).

Results and Discussion

Table 1 gives means with standard errors for age at fertile service, age at calving and weight of male & female calves for I to XII lactations. Age at fertile service increased from 42.91 ± 0.72 months for the Ist lactation cows to 196.50 ± 6.16 months for the XIIth lactation cows. Similarly age at calving increased from 52.19 ± 0.82 months to 207.00 ± 9.00 months for the same period. Weight of male calves ranged between 22.29 ± 1.19 kg (IInd lactation) to 29.50 ± 5.50 kg (XIIth lactation). Similarly, weight of female calves ranged between 21.02 ± 3.71 kg (Ist lactation) to 23.46 ± 0.56 kg (XIIth lactation).

Table 2 gives service period, gestation length, inter-calving period, duration of lactation and milk yield for I to XII lactations. Service period ranged between 112.06 ± 9.44 days (VIth lactation) to 259.00 ± 0.08 days (XIIth lactation) (Fig. I). Gestation period ranged between 283.47 ± 6.62 days (IIIrd lactation) to 293.00 ± 7.43 days (XIIth lactation). Intercalving period varied between

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TABLE 1: Effect of parity on age at fertile service, age at calving and weight of calf-born in Gir cattle (Mean±S.E.).

Lactation No.	Age at fertile service (months)	Age at calving (months)	Weight of calf (kg)	
			Male	Female
I	42.91±0.72 (114)	52.19±0.82 (120)	23.68±4.50 (61)	21.02±3.71 (52)
II	58.66±0.93 (96)	68.10±1.07 (97)	22.29±1.19 (45)	21.49±0.49 (50)
III	72.36±2.49 (78)	81.86±1.27 (79)	22.81±0.44 (42)	21.76±0.39 (40)
IV	85.42±1.33 (72)	94.74±1.03 (71)	24.01±3.52 (47)	23.21±8.88 (30)
V	97.54±4.22 (53)	106.92±1.49 (53)	24.45±10.93 (31)	21.84±5.08 (29)
VI	109.63±2.14 (40)	119.88±2.11 (40)	24.83±0.71 (31)	22.86±0.74 (19)
VII	121.68±2.32 (28)	130.25±2.58 (28)	25.17±0.63 (21)	21.83+0.87 (16)
VIII	132.76±7.19 (21)	141.41±9.28 (21)	24.92±2.69 (13)	22.54±0.86 (13)
IX	143.66±5.25 (12)	152.70±4.09 (12)	24.63±6.60 (12)	22.09±7.41 (13)
X	155.25±7.32 (4)	164.75±7.52 (4)	25.90±12.44 (7)	22.47±14.22 (7)
XI	174.33±14.63 (3)	183.32±12.96 (3)	26.25±1.56 (4)	22.11±12.74 (6)
XII	196.50±6.16 (2)	207.00±9.00 (2)	29.50±5.50 (2)	23.46±0.56 (5)
Overall	(523)	(530)	24.88±0.49 (316)	22.22±0.24 (285)

Figures in parentheses indicate number of observations.

403.55±11.03 days (VIth lactation) to 475.66±6.82 days (Ist lactation) (Fig. II). Duration of lactation and milk yield were lowest (252.88±12.14 days and 1254.80 ±169.74 kg) in the VIth and XIth lactations respectively. Whereas, they were highest (313.54±10.46 days and 1633.80±122.59 kg) in the Ist & IIIrd lactations respectively (Figs. III and IV).

Table 3 presents the analysis of variance for the effect of parity on weight of male and female calves, gestation length, service period, intercalving period, duration of lactation and milk yield in Gir cattle. Parity significantly ($P<0.01$) influenced the service period, intercalving period and days in milk whereas it had no significant effect on weight of calf (male & female), gestation length and milk yield.

Ponshke (1969), Kodagali (1980) and Velhankar (1973) have reported the age at first fertile service as 1007.95±22.56 days; 34.90±5.63 months and 978.66± 28.73 days respectively. These values are lower than the findings of the present study. This may be due to different locations and animals involved in the study and different animal husbandry conditions followed. Ponshke (1969) has

TABLE 2: Effect of parity on service period, gestation length, intercalving period, duration of lactation and milk yield in Gir cattle (Mean±S.E.)

Lactation No.	Service period (days)	Gestation length (days)	Intercalving period (days)	Diuration of lactation (days)	Milk yield (Kgs)
I	180.89 ±7.29 (77)	284.56 ±0.74 (12)	475.66 ±6.82 (77)	313.54 ±10.46 (118)	1537.76 ±20.36 (114)
II	164.94 ±8.65 (79)	284.64 ±0.89 (81)	465.40 ±8.08 (80)	292.01 ±8.98 (102)	1456.95 ±20.89 (101)
III	132.32 ±8.16 (79)	283.47 ±6.62 (62)	429.15 ±8.80 (79)	286.60 ±10.25 (87)	1633.80 ±122.59 (85)
IV	138.38 ±9.79 (59)	286.67 ±1.01 (56)	426.09 ±10.51 (58)	278.33 ±10.59 (80)	1581.17 ±71.58 (77)
V	126.18 ±10.45 (50)	284.62 ±0.92 (47)	404.50 ±14.37 (48)	273.43 ±14.37 (64)	1498.41 ±80.24 (64)
VI	112.06 ±9.44 (35)	285.33 ±1.07 (39)	403.55 ±11.03 (33)	252.88 ±12.14 (52)	1419.00 ±81.31 (52)
VII	127.24 ±11.52 (29)	284.74 ±2.09 (27)	408.46 ±13.57 (28)	255.86 ±16.01 (36)	1460.62 ±113.91 (36)
VIII	143.41 ±11.76 (24)	285.19 ±1.70 (26)	425.47 ±14.97 (23)	253.66 ±16.14 (30)	1512.42 ±104.43 (30)
IX	144.28 ±16.64 (14)	283.89 ±2.64 (29)	430.92 ±16.20 (14)	266.82 ±19.70 (23)	1370.43 ±128.40 (23)
X	151.66 ±21.07 (9)	284.30 ±10.79 (10)	434.37 ±14.70 (8)	286.14 ±40.12 (14)	1297.16 ±160.76 (14)
XI	161.29 ±19.98 (7)	285.14 ±2.56 (7)	421.50 ±37.08 (6)	261.20 ±23.86 (10)	1254.80 ±169.74 (10)
XII	259.00 ±0.08	293.00 ±7.43	—	278.67 ±48.42	1422.16 ±305.71
Overall	153.47 ±11.03 (467)	285.46 ±0.66 (401)	429.55 ±7.09 (454)	274.92 ±5.31 (622)	1453.72 ±39.92 (612)

Figures in parentheses indicate number of observations.

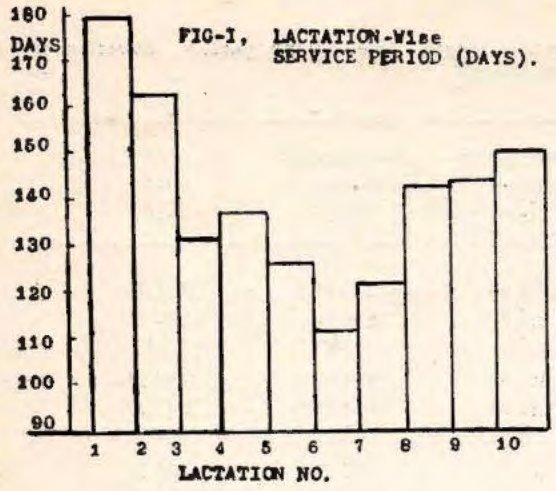

FIG-I, LACTATION-Wise SERVICE PERIOD (DAYS).

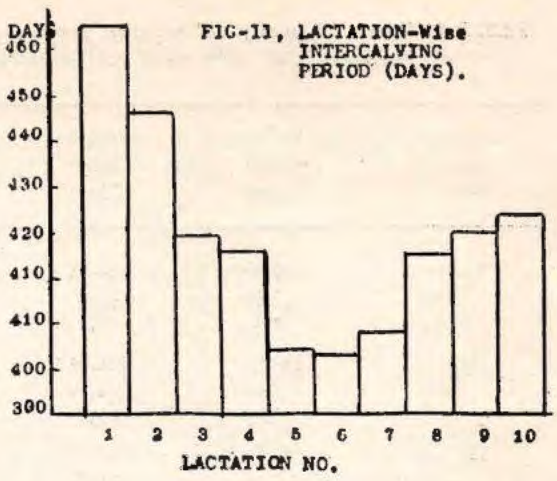

FIG-II, LACTATION-Wise INTERCALVING PERIOD (DAYS).

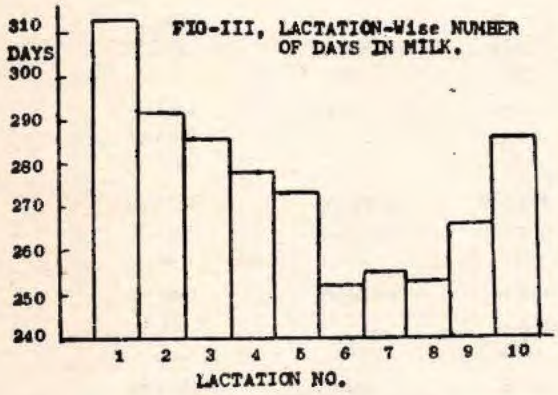

FIG-III, LACTATION-Wise NUMBER OF DAYS IN MILK.

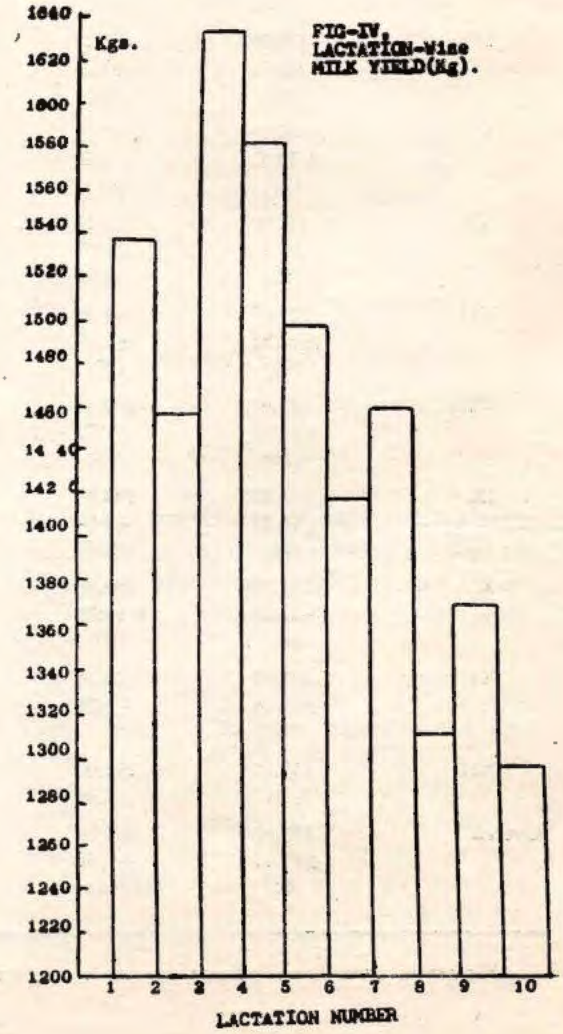

FIG-IV, LACTATION-Wise MILK YIELD(Kg).

reported the mean gestation period of 287.86 days for the 228 observations studied. This is comparable to the results of the present study. Pathak (1967) and Dange (1969) have also reported similar gestation periods of 283.30±0.14 days and 285.00±4.5 days respectively.

Anantkrishnan and Lazarus (1953) and Kodagali (1980) have reported the average birth weight of Gir calves as 49.3 lbs (male: 50.9 lbs and female 47.9 lbs) and 21.09±1.76 kgs, respectively. These observations are in agreement with the findings of the present study. Kodagali (1980) has reported the age at first calving as 44.20±5.63 months based on 284 observations. This is slightly higher than 42.91±0.72 months observed in the

TABLE 3. Analysis of variance to study the effect of parity on different parameters in Gir cattle.

Parameter	Source of variation	D.F.	M.S.S.	Significance
Weight of male calf	Between lactation	9	38.72	N.S.
	Within lactation	300	23.41	N.S.
Weight of female calf	Between lactation	9	34.28	N.S.
	Within lactation	264	29.80	N.S.
Gestation length	Between lactation	9	45.89	N.S.
	Within lactation	429	439.72	N.S.
Service period	Between lactation	9	23441.57	**
	Within lactation	445	4832.30	**
Intercalving period	Between lactation	9	26550.75	**
	Within lactation	438	5555.15	**
Duration of lactation	Between lactation	9	23621.51	**
	Within lactation	596	10191.84	**
Milk yield	Between lactation	9	532831.50	N.S.
	Within lactation	596	534262.00	N.S.

** Significant at $P < 0.01$ level
NS = Non-significant.

present study. This may be due to less number (114) of observations.

Basu *et al.* (1979) have reported mean service period of 68.07±23.29, 90.60±24.32 and 87.0±25.47 days in Sahiwal, Tharparkar and Red Sindhi cows which is higher than the findings of the present investigation. This may be due to genotypic and environmental differences in the animals studied. They have also reported the calving interval to range between 350.22±56.39 to 410.46±34.62 in Sahiwal, Tharparkar and Red Sindhi cows which is lower than the observations of the present study.

Mean lactation length and milk yield have been reported to range between 271-308 days and 1345.33—1799.08 kg (Anon, 1973). These observations are comparable to findings of the present investigation.

Conclusions

It can be surmised from the results that reproductive and productive efficiency is achieved at or after the 3rd, lactation in Gir cattle. Best results could be obtained upto the 6th lactation in these animals. Further, parity did not significantly influence the weight of calves, gestation length and milk yield for Ist to XIIth lactation studied in the home tract of this breed.

Acknowledgement

The authors are thankful to the Principal, Gujarat Veterinary College, Anand and the authorities of Virani Goshala, Mangrol for encouragement and facilities provided for undertaking the present study.

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Reproductive Performance Of Kankrej And Its F_1 Cross-breds With Jersey And Holstein

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ABSTRACT

Age at first calving, inter-calving period, dry period and number of inseminations/conception were studied for Kankrej and its F_1 cross-breds with Jersey and Holstein in a herd of Livestock Research Station, Anand. Average age at first calving was 1579.26±18.36, 835.76± 12.79 and 887.33+24.27 days in Kankrej, JK cross-breds and HK cross-breds respectively. The inter-calving period in the respective breed group was 450.22± ±20.15, 397.15±14.42 and 433.80±24.56 days. Dry period was significantly less in both the eross-breds (76.0 days) as compared to parental Zebu breed (159.0 days). Average number of inseminations required per conception were 3.62, 2.58 and 4.76 in Kankrej, JK cross-bred, and HK cross-breds respectively. The results revealed that age at first calving is redueed sigmificantly by introduction of exotic germ plasm. Jersey cross-breds were found to be far better to Holstein cross-breds in their reproductive efficiency.

* * *

Efficient reproduction is *Sine qua non* for economical milk production. It needs no documentation to state that the most of the traits related to reproduction are largely influenced by non-genetic factors viz., management, nutrition, sexual health etc. After implementation of cross-breeding on a national scale large number of reports (Sharma and Bhatnagar, 1975; D'Souza *et al.*, 1978; Manickam *et al.*, 1978; Kaushik *et al.*, 1979; Bhatnagar *et al.*, 1979 and Kaikini *et al.*, 1981) have shown that cross-breds have better reproductive performance than the parental Zebu breeds. As the work of cross-breeding of Kankrej with Jersey and Holstein is of recent origin in Gujarat, the present investigation was undertaken to study the extent of improvement in certain reproductive traits in F_1 cross-breds.

Materials and Methods

The data on age at first calving, inter-calving period, dry period and inseminations/conception were compiled from the records of Livestock Research Station, Anand from the year 1979 to 1985. The herd was maintained entirely by stall feeding with green fodders and concentrate. The detection of heat in the herd was carried out twice in a day by a closed supervision of an experienced technical person. Inseminations were done using frozen semen of high quality bulls. The analysis of data was carried out using standard procedures as described in Snedecor and Cochran (1971).

Results and Discussion

Mean values of age at first calving, inter-calving period, dry period and inseminations/conception have been shown in Table 1.

TABLE 1: Mean values of different reproductive traits in Kankrej, Jersey × Kankrej and Holstein × Kankrej F_1 cross-breds.

Reproductive traits	Kankrej	Jersey × Kankrej	Holstein × Kankrej
Age at first calving (days)	1579.26[a] ±18.36 (45)	835.76[b] ±12.79 (50)	887.33[c] ±24.27 (44)
Inter-calving period (days)	450.22[a] ±20.15 (125)	397.15[b] ±14.42 (48)	433.80[ac] ±24.56 (32)
Dry period (days)	159.00[a] ±14.50 (125)	76.08[b] ±10.13 (48)	75.80[b] ±9.32 (32)
Inseminations/ conception	3.62[a] ±0.44 (279)	2.58[b] ±0.21 (102)	4.76[c] ±0.65 (80)

* Figures in the parentheses indicate number of observations.

** Figures bearing common superscript do not differ significantly from each other.

Age at first calving:

From the table it can be seen that the age at first calving (AFC) was significantly lower in both the cross-bred breed groups than that of Kankrej. The reduction in AFC was to the extent of 52.2% in Jersey × Kankrej (JK)F_1 and 43.67% in Holstein × Kankrej (HK)F_1 cross-breds. The reduction in AFC due to cross-breeding of indigenous cattle with exotic dairy breed has also been reported by Bhatnagar and Sharma, 1976; Manickam *et al.*, 1978 and Kaushik *et al.*, 1979, but the extent of reduction was lesser than that observed in the present study. Difference in age at first calving of two cross-bred breed groups was highly significant ($P < 0.01$).

It can be seen further from the Table that the inter-calving period in Jersey cross-breds was significantly low (397.15 ±14.42 days) as compared to Kankrej (450.22±20.15) and Holstein × Kankrej (433.80±24.56 days) cross-breds. The results thus showed that Jersey cross-breds were superior to the Holstein cross-breds in their reproductive efficiency. The long inter-calving period of HK cross-breds was mainly due to long service period accompanied with extended lactation period. Verma *et al.* (1983) also observed long inter-calving period in Holstein × Hariana than in Jersey × Hariana cross-breds.

As far as dry period is concerned it can be noted from the Table 1 that both the cross-bred breed groups had almost indentical dry period (76.0 days) and it was significantly lower than that of Kankrej (159.00±14.50 days).

TABLE 2. Average Inseminations/Conception in Kankrej, Jersey × Kankrej and Holstein × Kankrej F_1 Cross-breds.

Year	Kankrej			Jersey × Kankrej cross-breds			Holstein × Kankrej cross-breds		
	Heifers	Cows	Pooled	Heifers	Cows	Pooled	Heifers	Cows	Pooled
1979-80	2.80(25)	1.00(2)	2.66(27)	—	—	—	—	—	—
1980-81	3.30(57)	1.64(11)	3.03(68)	—	—	—	—	—	—
1981-82	—	4.63(54)	4.63(54)	1.25(4)	—	1.25(4)	2.50(4)	—	2.50(4)
1982-83	—	5.11(52)	5.11(52)	2.68(19)	2.00(5)	2.54(24)	5.54(13)	2.00(3)	4.87(16)
1983-84	—	2.65(52)	2.65(52)	1.64(11)	1.74(19)	1.70(30)	5.00(14)	3.38(8)	4.41(22)
1984-85	—	3.04(26)	3.04(26)	4.59(17)	2.52(27)	3.32(44)	4.82(17)	5.43(21)	5.16(38)
Overall	3.14(82)	3.82(197)	3.62(279)	2.98(51)	2.17(51)	2.58(102)	4.87(48)	4.90(32)	4.76(80)

Figures in the parentheses indicate number of animals in each year.

TABLE 3: Percentage Distribution of Inseminations/Conception in Kankrej, Jersey×Kankrej and Holstein×Kankrej F_1 cross-breds.

Insemination No.	Kankrej		J K Cross-breds		H K cross-breds	
	Heifers	Cows	Heifers	Cows	Heifers	Cows
First	26.25	14.80	40.00	50.00	12.13	12.50
Second	23.75	14.80	20.00	25.00	16.67	12.50
Third	21.25	12.90	14.00	8.00	20.00	18.90
Fourth	10.00	16.60	14.00	6.00	10.00	12.50
>Four (Repeaters)	18.75	40.00	12.00	17.00	40.00	43.70

Insemination/Conception:

The year-wise number of inseminations required per conception by Kankrej, Jersey×Kankrej and Holstein × Kankrej have been shown in Table 2. Percentages of cows and heifers conceiving with one, two, three, four and more than four inseminations have been presented in Table 3.

Perusal of the data in Table 2 shows that the heifers on an average required more number of inseminations than the cows. The average number of inseminations per conception were 3.14, 2.98 and 4.87 in heifers and 3.82, 2.17 and 4.90 in cows, respectively, for Kankrej, JK cross-breds and HK cross-breds. The average number of inseminations per conception in Kankrej (3.62 ± 0.44) is comparatively higher than that reported by Sharma and Bhatnagar (1975) for Tharparkar (2.4 ± 0.11) and Sahiwal (2.7 ± 0.11), Singh and Singh (1970) for Hariana (2.18) and Manickam *et al.* (1978) for Sindhi (2.2 ± 1.6) cattle. The average number of Inseminations/conception in JK cross-breds (2.58 ± 0.11) is also slightly higher than that reported by Kaushik *et al.* (1979) for Jersey× Hariana (2.27) and Manickam *et al.* (1978) for Jersey×Sindhi (1.8 ± 1.3) cross-breds. The higher number of inseminations/conception in the present study can be attributed to the fact that all the animals of the herd were included in the present study considering that repeat breeders were also the part of the herd.

The average number of inseminations per conception observed in the present study for Holstein×Kankrej are quite high and alarming, indicating thereby that though very good in production efficiency these cross-breds are very poor in their reproductive efficiency as compared to Jersey cross-breds. Therefore, before recommending any one of the cross-breds, the milk production efficiency in a calving interval time has to be given due consideration. Poor conception rate in Holstein cross-breds as compared to Jersey cross-breds has also been reported by several workers (D'Souza *et al.*, 1978; Kaikini *et al.*, 1981; Verma *et al.*, 1983 and Madhavan *et al.*, 1984) but it was not as poor as in this study.

From the Table 3 it can be seen that percentage of animals conceiving to first insemination were the highest in Jersey cross-breds and the lowest in Holstein cross-breds. At the same time it can also be noted from the table that percentage of animals requiring more than four inseminations were quite high in Holstein cross-breds followed by in Kankrej. The percentage of such animals

in Jersey cross-breds were fairly low.

From the results of inter-calving period and inseminations per conception it is evident that the Jersey cross-breds are far superior to Holstein cross-breds in their reproductive efficiency. From the overall fertility results it is suggested that in Jersey × Kankrej cross-breds, animals requiring more than three inseminations and in Holstein × Kankrej, animals requiring more than five inseminations should be classed as "Repeat Breeders" and after thorough examination should be disposed off from the herd. Looking to the overall fertility status of the herd it is suggested that multi-disciplinery investigations should be undertaken to find out the real cause for poor fertility status especially in Holstein × Kankrej cross-breds.

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CASE REPORTS

IJAR 7: 2: 85-87, 1986

Clinical Feminisation in a Bullock

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The following report is based on a clinical feminisation condition met in a bullock at one of the Gynaecological Camp held on 1-3-81 at Gadat in Gujarat State.

A twelve year old bullock (Fig-1) of Kankrej breed with hypertrophy of mammary tissue and enlarged teats was presented for examination. The animal had history of burdizzo castration on two, three occasions and the gonads were missing in the scrotal sac. The work efficiency of the animal was not markedly affected but the unsightly appearance (feminisation) was the problem.

For a clinician components of scrotum, its contents free of obvious abnormalities, size, consistency etc., are only at his disposal for diagnosis along with rectal exploration.

In the present case detailed clinical examination revealed obvious signs of feminisation, gynaecomastia, hypertrophy of mammary tissue (Fig.1), enlarged teats (Fig-2), metastases extra scrotal (Fig 3), inguinal canal, abdominal cavity lymphnodes pre-femoral (sub-iliac) (Fig. 4) and perineal skin involvement with melanotic discoloration (Fig. 3) were present. On histopathological examination from the biopsy material collected, SCT in retained testes with metastasing condition could be diagnosed.

Tumours of testes are rare in bulls. Clinically diagnosed tumours are extremely rare. Most of them are observed at necropsy in aged bulls. (Jubb and Kennedy, 1970). Most domestic species are castrated before they reach the cancer age. The testes may be involved in disease process in a number of ways. It can be the primary site of clinical abnormality in tumour formation (cryptorchidism). Three types of testicular tumours are recognised viz., seminomas (S), Sertoli Cell Tumour (SCT) and Interstitial Cell Tumour (ICT).

SCT tumour occurs bilaterally. The incidence is more in cryptorchid testes. (Lipowitz *et al.*, 1973). Firm mass is felt in 10% and malignancy signs of feminisation are seen in 25% cases. This is said to be the most clinically dramatic

No.	Type	Cells involved	*Effects*
I	S	Spermatogenic cells	Sperm production
II	SCT	Nurse cells	Infertility
III	ICT	Leydig cells	Testicular degeneration due to compression and steroid production.

Fig. 1

Fig. 2

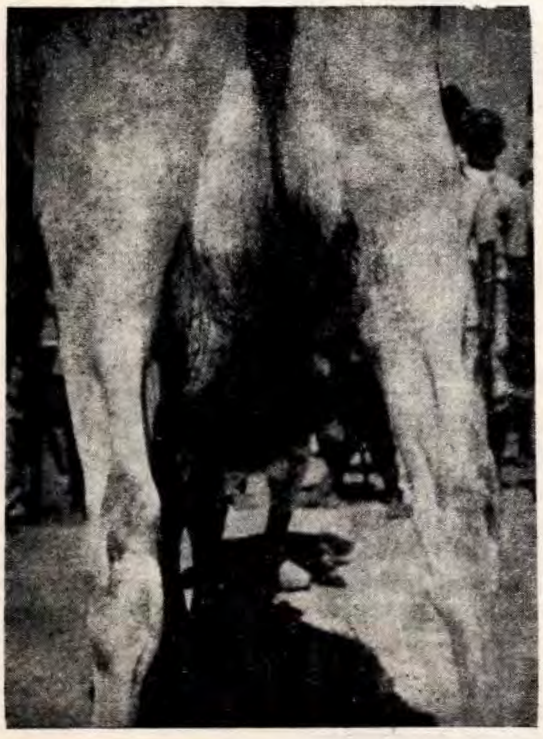

Fig. 3

Fig. 4

tumour which is endocrinally an active one. The quantity of oestrogens produced is related to the size of tumour developing from the retained testes and the metastasis following clinical signs of feminisation. The signs observed are undoubtedly due to oestrogens now known to be produced by SCT tumours.

As this was an advanced case neither surgical nor medicinal treatment was undertaken. A clinical record and report has been made since this condition is met very rarely.

Acknowledgement

Thanks are due to the DAHO, Surat; Veterinary Officer, Vyara and FAVC, Gadat for all the help.

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IJAR 7: 2: 88-89, 1986

Use Of Lutalyse In The Expulsion Of Mummified Foetus

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ABSTRACT

Lutalyse could be used successfully in treating a case of mummified foetus in cattle.

* * *

The most common practise to expel a mummified foetus is by using oesrogens either alone or in combination with pituitrin or Dexamethasone (Roberts, 1956; De *et al.*, 1965 and Rao and Murthy, 1978). In Modern Veterinary Practice, only very few reports are available enlightening the usefulness of prostaglandins (Cooper *et al.*, 1976; Jenkins *et al.*, 1977).

Case History

A non-descript heifer was brought to the clinic on 27-6-86 (Case No. 685) with a history that it failed to deliver the calf, though the animal was inseminated about 18 months back.

Diagnosis and Treatment

On palpation per rectum, the foetus was found to be located deep on the abdominal floor with contracted uterus tightly enclosing the foetus. Neither the fluctuation of a gravid uterus nor the cotyledons could be felt. The fremitus was absent. Failure of udder development was noticed. Based on the history and clinical findings it was diagnosed as a case of mummified foetus.

A single intramuscular injection of 5 mg of Lutalyse (Upjohn co., Kalamazoo, USA) was given. Though the ejection of the foetus could not take place, a brownish chocolate coloured exudate oozing from the uterus was observed for 3 days. Then a second dose was tried. After a lapse of a further 72 hours, rectal examination revealed that the foetus has advanced into the birth canal and the same was removed by applying lubrication and traction (Jackson *et al.*, 1977).

The entire foetus was shrivelled up (Fig). The foetal membranes were dark-brown in colour, thick and were found closely wrapping the foetus.

In the present case it was not possible to establish the cause of foetal death and mummification. The second dose of Lutalyse and forced traction was necessary to extract the foetus as the case was of a long standing nature.

Fig 1: Mummified foetus

Acknowledgement

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IJAR 7: 2: 90-91, 1986

Uterine Torsion In A Goat - A Case Report

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ABSTRACT

A rare case of post-cervical right side torsion of uterus in a goat is reported and the method of successful delivery per vaginum is discussed.

* * *

Uterine torsion in bovines is usually a complication of late first-stage or early second-stage of labour which, if not properly diagnosed and rationally treated, may lead to the death of the mother or the foetus or both. This condition occurs less frequently in ewe and goat than in the cow (Farman,1965; Moar,1965; Tallantirr 1965; Roberts, 1971 and Arthur *et al.*, 1982). The treatment of uterine torsion consists of caesarian section or rapidly rolling the doe in the direction of the torsion while stabilizing the vagina (Bowen, 1981). Successful correction of uterine torsion in a goat by rolling the doe with extra abdominal pressure and effecting per vaginum delivery is reported in the present communication.

Case History

A local non-descript goat approximately of two years of age, was presented to the specialised unit of Punjab Agricultural University Veterinary Clinics with the history that the animal, in her full term, exhibited all signs of parturition since previous evening but has failed to deliver kids.

Clinical Examination and Diagnosis

The animal was showing straining or expulsive efforts. It was completely anorectic but took plenty of water. Vaginal speculum and digital examination revealed twisting of vagina towards right side (clock-wise). Due to the twisted, narrow vagina, the foetus or cervix could not be palpated. The case was diagnosed as post-cervical right side torsion of uterus.

Treatment

Following the restraint of the animal in right lateral recumbency, the goat was given one roll towards right side with slight pressure on left flank with the help of both hands. The complete detorsion was achieved with only one roll. Per vaginal examination indicated that cervix was palpable and only one finger open. It was decided to wait for some time. Therapy consisting of Dexona[1]—2 ml and Cemizol[2]—5 ml was given intramuscularly. Complete dilatation of the cervix was achieved by gradual manuplation after two hours. Water begs presented in the genital passage were ruptured and a dead female kid with bilateral knee flexion was delivered through traction after correction. Oxytocin[3] —25 I.U. was administered I/M following the removal of foetal membranes manually

1 Dexona (Dexamethasone 4 mg/ml) — Cadila, Ahmedabad.
2 Cemizol (Analgin 500 mg/ml) — IDPL, Rishikesh.
3 Oxytocin (5 I.U./ml).—BMG Pharma. Calcutta.

Discussion

Exact aetiology of uterine torsion is still obscure. Various predisposing factors in uterine torsion in goats could be falling, rolling or inadequate exercise (Bowen, 1981). The low incidence of this malady in ewes could be either due to sublumber attachment of mesometrium rather than subilial as in cows (Arthur *et al.*, 1982) or due to higher frequency of twin pregnancy, which might be attributed to greater curvature of the gravid uterus (Roberts, 1971 and Arthur *et al.*, 1982). In the present case torsion may be attributed to the presence of one foetus in one horn resulting in imbalance of the uterus.

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IJAR 7: 2: 92-93, 1986

Post-Partum Uterine Prolapse In A Goat - A Case Report

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ABSTRACT

A case of post-partum uterine prolapse in a goat is reported and the method of successful reposition is discussed.

* * *

Utero-vaginal prolapse occurs in all species of domestic animals but is observed most frequently in bovines and rarely in goats. It is a complication of the third stage of labour in cow and ewe (Arthur *et al.*, 1982). Uterine prolapse is rare but may occur in dose exhibiting persistant straining following kidding, prolonged difficult birth or retained placenta. A case of utero-vaginal prolapse treated successfully is placed on record.

Case History

A local non-descript goat approximately of 4 years age, was brought to the specialized unit of Punjab Agricultural University Veterinary Clinics with the history that it gave birth to one kid two days ago. Kidding was normal and the foetal membranes were expelled the next day morning. Suddenly in the evening on the same day, there was complete eversion of the uterine mass.

Clinical Examination

Animal was depressed and anorectic. The protruded uterine mass was soiled and had several small contusions and lacerations on it (Fig. 1). Part of the foetal membranes was also adhered.

Treatment

The animal was given epidural anaesthesia using lignocaine (2%) 4 ml. The protruded uterine mass was cleaned with cold water and adhered portions of foetal membranes were detached slowly. Oxytocin 15 I.U. was administered locally in the uterine wall to reduce the volume of the uterus. Then the animal was put in dorsal recumbency and hind quarters were slightly elevated. The uterus was lubricated with M & B antiseptic cream and it was reposed taking all the necessary precautions described by Roberts (1971). No vulvar sutures were applied.

Drug therapy consisting of Otcim[1] 10 ml, Anthisan[2] 5 ml, Cemizol[3] 5 ml, Dexona[4] 2 ml, intramuscularly and normal saline solution 1 litre intravenously were given on the day of treatment. Antibiotic therapy was continued subsequently for another 3 dyas.

Discussion

Uterine prolapse is an emergency which needs immediate treatment, otherwise the interference in the blood supply to

1 Otcim — (Oxytetracycline 50 mg/ml) — IDPL, Rishikesh.
2 Anthisan — (Mapyramine Maleate 5%) — May and Baker, Bombay.
3 Cemizol — (Analgin 500 mg/ml) — IDPL, Rishikesh.
4 Dexona — (Dexamethasone 4 mg/ml) — Cadila, Ahmedabad.

Figure: 1 Uterine Prolapse in a Goat.

the prolapsed tissue may result in oedema and cyanosis which may eventually lead to gangrene of the uterus. As symptoms of shock often accompany prolapse of the enlarged oedematous uterus, so dexamethasone and normal saline solution were given parenteraly. Histamine is expected to be released in large quantity in severe degree of prolapse particularly those associated with injury. This would bring about systemic disturbances hence, mapyramine maleate was given intramuscularly. For reducing haemorrhage and size of the prolapsed mass oxytocin was given locally in the uterine wall. Analgesic was given to relieve the animal from pain.

In the present case uterine prolapse may be due to the presence of undetached portion of foetal membranes. However, Arthur *et al.* (1982) believed that uterine inversion and prolapse were associated with the onset of uterine inertia during the third stage of labour when a portion of detached afterbirth occupied the birth canal and protruded from the vulva.

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THESIS ABSTRACTS

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Studies On Seminal Characteristics, Biochemical And Enzymatic Constituents, Freezability, Enzyme Leakage And Fertility In Surti Buffalo Bulls

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The present investigation was undertaken on 4 Surti buffalo bulls during a period of one year (wet, hot & cold seasons) at the Department of Veterinary Obstetrics & Gynaecology, Gujarat Veterinary College, Anand. The studies included: evaluation of seminal characters, assay of biochemical & enzymatic constituents, freezability, efficacy of different extenders, leakage of enzymes, fertility and their correlations. The seasonal influence was also studied.

Ranking of Bulls:

Bulls were ranked, based on overall performance, semen characters, initial fructose, LDH activity, freezability and fertility. Two bulls could be grouped under excellent sexual function and two in normal sexual function. Libido and serving behaviour in these bulls were within normal limit.

Seminal Attributes:

Based on 276 semen ejaculates collected once at 5 days interval, the mean values for various seminal attributes were: ejaculate volume 2.80±0.06 ml, colour & constistency 2.95±0.05, mass activity 2.90±0.09, pH 6.81±0.005, individual motility 82.59±0.31 %, sperm count per ml 1013.68±14.95 million, sperm count per ejaculate 2898.77±84.65 million, live sperm 87.09±0.26 %, dead sperm 12.87±0.26 % and abnormal sperm 7.58±0.26 %.

Biochemical and Enzymatic Constituents:

Biochemical and enzymatic constituents were estimated in 96 semen/seminal plasma samples obtained at fortnightly interval. The mean values were: initial fructose 555.37±13.79 mg %, citric acid 370.33±8.18 mg %, total protein 4.42±0.07 g %, total solids 5.49±0.11 %, sodium 182.77±3.47 mg %, potassium 83.13±2.87 mg %, GOT 51.50±1.60 μ mole/lit, GPT 14.51±0.97 μ mole/lit, AKP 590.34 ±8.70 KAU/100 ml, ACP 252.32±5.06 KAU/100 ml and LDH 387.05±7.47 IU/litre.

The 'F' test analysis for the effect of bulls, seasons and bulls×seasons interactions revealed the differences between bulls to be highly significant ($P<0.01$) for volume, colour & consistency, mass

activity, individual motility, sperm count per ml and per ejaculate, abnormal sperm %, GOT, AKP and potassium. Bull effect was significant ($P<0.05$) for live & dead sperm %, initial fructose, citric acid and LDH activity. The influence of seasons was highly significant ($P<0.01$) for colour & consistency, abnormal sperm %, citric acid, total solids, sodium, potassium, GOT and GPT, and significant ($P<0.05$) for mass activity, live & dead sperm %, AKP and ACP. The interaction effect was highly significant for LDH and significant for sodium. Semen quality in general, was poor in wet season as compared to cold & hot seasons.

Freezability of ejaculates:

Based on 136 semen ejaculates frozen for fertility trials, the overall acceptable freezability ejaculates (post-thaw motility 35% & above) were 122 (89.71%), whereas the poor or unfreezable ejaculates averaged 14 (10.29%). Of the 122 ejaculates, excellent, good and moderately freezable ejaculates were 52 (38.24%), 39 (28.68%) and 31 (22.79%), respectively. The bulls under study showed marked variations in their ability to provide acceptable freezability ejaculates.

Revivability of Spermatozoa:

Twelve ejaculates, monthly once from each of 4 bulls, frozen in TFYG, EYCG and LYG diluents at the rate of 1:10 dilution under split ejaculate technique revealed the effect of dilutors to be non-significant for variation in spermatozoal motility at different stages of freezing. However, variation in sperm motility was highly significant ($P<0.01$) between bulls and between seasons with the overall pre and post freeze (0 hr and 7th day) motility of 77.08 ± 0.72, 44.90 ± 1.09 and 42.43 ± 1.04 per cent, respectively. The values were of low order at all the stages of freezing during wet season. The freezability of semen in TFYG alone ranged between 45.00 to 5[illegible].70 % among the 4 bulls. All two-way interactions between bulls, seasons, dilutors and stages of freezing were non-significant.

Enzymes leakage:

Forty eight ejaculates studied for enzymes leakage using split ejaculate technique at the rate of 1:10 dilution revealed the differences in the overall mean GOT-GPT, AKP-ACP and LDH enzymes activity in pre and post freeze seminal plasma to be highly significant ($P<0.01$). The pre and post freeze GOT, GPT, AKP, ACP and LDH levels were 20.88 ± 0.81 & 33.07 ± 1.14 μ mole/lit., 7.05 ± 0.32 & 11.44 ± 0.47 μ mole/lit., 68.02 ± 3.72 & 94.32 ± 4.50 KAU/100 ml, 31.60 ± 1.74 & 53.22 ± 2.46 KAU/100 ml and 114.61 ± 5.77 & 156.24 ± 6.57 IU/lit., respectively. The effect of dilutors was highly significant ($P<0.01$) for AKP release, the levels being in ascending orders for TFYG, EYCG and LYG diluents. The influence of season was highly significant for the levels of GOT, AKP and ACP, significant for GPT and non-significant for LDH. Greater values of GOT and GPT were observed in wet seasons and values were of low order for AKP and ACP in hot season. The differences between bulls were highly significant ($P<0.01$) for levels of GOT, AKP and significant ($P<0.05$) for LDH. The interaction effects viz., period × season for GOT & AKP, bull × season for GPT & AKP and dilutor × season for AKP were only significant amongst all the two-way interactions studied.

Fertility Trials:

The overall fertility rate with 2995 frozen semen inseminations in 2195 Surti

buffaloes of Panchmahal District was 40.13%, requiring 4.05 inseminations/ conception. The four bulls varied in their fertilizing ability from 37.92 to 44.65%. Insemination with semen frozen in TFYG diluent showed significantly ($P<0.05$) higher fertility results (43.62%) as compared to EYCG (40.22%) and LYG (36.52%) diluents. The overall fertility rates for semen frozen in low and high breeding seasons and used for AI only in high breeding season, were 39.37 and 41.00 %, respectively. This indicated equally good fertility in summer as well as in winter frozen semen.

Based on 1500 (808 fresh & 692 repeat) frozen semen inseminations in 808 Surti buffaloes of Bedva (Anand), the fresh inseminations conception rate was 30.88% and overall conception rate was 69.69%. A highly significant difference was observed between months for fresh as well as overall inseminations conception, being lowest (5.66% & 4.62%) in June and highest in months of November (47.06%) and December (44.26%). The monthly THI was significantly and negatively correlated with monthly conception rates for fresh (−0.678) and overall (−0.693) inseminations. On an average 3.24 & 2.71 inseminations per conception were required for fresh and overall conception. The first inseminations conception rates between 17 Surti bulls used at random, varied highly significantly (9.09 to 61.11%). In general, conception rate for fresh inseminations performed in high breeding season (Sept.-Feb.; 38.03%) was 2½ times greater than that in low breeding season (Mar.-Aug.; 14.69%).

Correlation Studies:

The correlation studies based on bull-wise seasonal means revealed ($P<0.05, \pm 0.576$; $P<0.01, \pm 0.708$) that *in seminal characters:* Ejaculate volume was correlated with sperm concentration per ejaculate (0.879). Colour & consistency was correlated with mass activity (0.915), pH (−0.616), sperm concentration per ml (0.843), sperm concentration per ejaculate (0.663), motility (0.689), live sperm %(0.548), dead sperm % (−0.546) and abnormal sperm % (−0.970).Seminal pH was correlated with mass activity (−0.707), individual motility (−0.602), sperm concentration per ml (−0.764), live sperm% (−0.666), dead sperm % (0.667) and abnormal sperm % (0.575). Mass activity was correlated with individual motility (0.765), sperm concentration per ml (0.861), sperm concentration per ejaculate (0.721), live sperm % (0.576), dead sperm % (−0.576) and abnormal sperm % (−0.859). Individual sperm motility was correlated with sperm concentration per ml (0.671), live sperm % (0.926), dead sperm % (−0.945) and abnormal sperm % (−0.725). Sperm concentration per ml was correlated with sperm concentration per ejaculate (0.609), live sperm % (0.598), dead sperm % (−0.620) and abnormal sperm % (−0.803). Sperm concentration per ejaculate was correlated with abnormal sperm % (—0.685). Live sperm % was correlated with dead sperm % (−0.995) and abnormal sperm % (−0.619). And dead sperm % was correlated with abnormal sperm % (0.615).

In biochemical & enzymatic constituents: Citric acid was correlated with ACP (0.568) & sodium (0.783). Total protein was correlated with total solids (0.639) & GOT (0.589). Total solids was correlated with sodium (0.533) & LDH (0.660). GOT was correlated with GPT (—0.830). ACP was correlated with sodium (0.722) & potassium (0.595). And sodium was correlated with potassium (0.503).

Between seminal characters and biochemical & enzymatic constituents: Ejaculate volume was correlated with initial fructose (0.633),

AKP (−0.615) & potassium (0.519). Colour & Consistency was correlated with total protein (0.636), total solids (0.685), GOT (0.519) and sodium (0.512). Seminal pH was correlated with GOT (−0.577) & GPT (0.531). Mass activity was correlated with total protein (0.592), total solids (0.539) & citric acid (−0.549). Individual motility was correlated with ACP (0.501) & potassium (0.509). Sperm concentration per ml was correlated with total protein (0.580). Sperm concentration per ejaculate was correlated with initial fructose (0.525) & AKP (−0.636). Live sperm per cent was correlated with GOT (0.544) & potassium (0.547). Dead sperm per cent was correlated with GOT (−0.524) & potassium (−0.511). Abnormal sperm per cent was correlated with total protein (−0.606), total solids (−0.573), GOT (−0.560), AKP (0.530), ACP (−0.529) and sodium (−0.526).

Between freezability, fertility and enzymes leakage: The fertility rate was correlated with the levels of post-freeze LDH (0.567), pre (−0.512) & post freeze (−0.608) AKP, sperm count per ml (0.545), initial seminal fructose (0.905) and with GOT (0.831), AKP (−0.928) & LDH (0.851) in neat seminal plasma. Pre-freeze motility was correlated with post-freeze motility (0.575), pre (0.899) & post freeze (0.868) GOT, pre (0.583) & post freeze (0.739) LDH and post-freeze ACP (0.505). Post-thaw motility was correlated with pre (0.690) & post freeze (0.728) LDH, pre-freeze GOT (0.526) and with GOT (0.844) & GPT (−0.690) in neat seminal plasma. Pre-freeze GOT was correlated with post-freeze GOT (0.935), pre-freeze GPT (−0.541), pre (0.643) & post freeze (0.641) ACP, post-freeze LDH (0.882) and sperm count per ml (0.650). Post-freeze GOT was correlated with pre (0.708) & post freeze (0.652) ACP, pre (0.641) & post freeze (0.785) LDH and sperm count per ml (0.608). Pre-freeze GPT was correlated with post-freeze GPT (0.708) and pre-freeze LDH (−0.516). Pre-freeze AKP was correlated with post-freeze AKP (0.983) & sperm count per ml (−0 633). Post-freeze AKP was correlated with sperm count per ml (−0.623). Pre-freeze ACP was correlated with post-freeze ACP (0.583) and pre-freeze LDH (0.640). Post-freeze ACP was correlated with post-freeze LDH (0.535). And pre-freeze LDH activity was correlated with post-freeze LDH activity (0.899) of frozen- thawed seminal plasma.

Conclusions:

The available literature revealed that the implimentation of AI in buffaloes has been posing problems in respect of semen quality, preservability, suitability of diluents, freezability and fertility of frozen semen. Relatively few reports deal with the use of suitable diluents for buffalo semen freezing based on eleborate enzymatic evaluations and actual fertility trials. From the results on the various facets of the present investigations, it is conclued that the evaluations of seminal characteristics and biochemical & enzymatic profiles have a great bearing and are useful means in indicating the quality of semen ejaculates, their freezability and fertility. The extent of various enzymes leakage viz., transaminases, phosphatases and dehydrogenase was very indicative of the freezability and fertility of the semen samples. In good freezability bulls the enzymes leakage was of minimum order when compared with the poor freezability bulls. Estimations for the profiles of LDH, GOT & AKP enzymes and initial seminal fructose content fairly indicate fertility status and can be used to predict fertility of frozen buffalo semen.

IJAR 7: 2: 98-99, 1986

Studies On Seminal Characters, Freezability And Fertility Of Cross-bred (K ×HF : K ×J) Bulls

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Ph.D. THESIS 1986

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The present investigation on "Studies on Seminal Characters, Freezability and Fertility of Crossbred Bulls" was undertaken during a period of one year (wet, cold and hot seasons) at the Department of Veterinary Obstetrics and Gynaecology, Gujarat Veterinary College, Anand. The study included evaluation of seminal characteristics, assay of seminal biochemical constituents, freezability, efficacy of various dilutors, cytomorphology and mensuration characteristics of sermatozoa, extracellular release of enzymes and effect of additives on semen quality and fertility.

Bulls were ranked depending on the freezability. The 8 bulls were divided into two groups viz., good and poor freezability having equal bulls of each breed/type.

Bulls from good freezability group showed significantly better libido, shorter reaction time, higher ejaculate volume, distinctly superior mass activity, higher sperm concentration/ml, lower dead and abnormal sperm count and higher electrical conductivity of semen than those from poor freezability group. Season had marked effect on pH, sperm concentration/ml, dead and abnormal sperm count.

The correlation studies revealed ($P<0.05$) that in seminal characters: Ejaculate volume was correlated with mass activity (0.875), sperm concentration (0.813), freezability (0.840), sperm head breadth (0.838), dead sperm count (−0.880) and abnormal sperm count (−0.828). Mass activity was correlated with sperm concentration (0.839) and freezability (0.838). Sperm concentration was correlated with dead sperm count (−0.947), abnormal sperm count (−0.938), freezability (0.834) and sperm head breadth (0.996). Dead sperm count was correlated with abnormal sperm count (0.985), freezability (0.826) and sperm head breadth (−0.954). Abnormal sperm count was correlated with freezability (−0.826). And freezability was correlated with sperm head breadth (0.889).

Bulls in good freezability group showed higher levels of phosphorus and sodium and lower levels of calcium, potassium, sulphur and chloride than bulls in poor freezability group. Correlation studies revealed that calcium was significantly and negatively correlated with ejaculate volume (−0.811).

The freezability of semen ranged between 57.61 to 69.97 % with an average of 62.71 % in good freezability group and between 40.75 to 49.76 % with an average of 44.91 % in poor freezability group. The overall average freezability was 53.81 %.

Mean dead and abnormal sperm count in good freezability group was significantly lower (29.69 % & 10.13 %) than in poor freezability group (39.97 % & 10.97 %, resp.).

In all the three parameters studied above, the effects due to groups, seasons, dilutors and periods were highly significant.

Mean sperm head length and total length of spermatozoa were significantly shorter in good freezability (9.54 & 70.22 micron) than in poor freezability group (9.59 and 70.96 micron), but mean sperm head breadth was significantly greater in good freezability than in poor freezability group (4.55 and 4.49 micron). In all the parameters, effect due to groups and seasons was significant.

Neither the freezability group nor season had significant influence on GOT, GPT, AKP and ACP levels in seminal plasma of neat semen.

GOT and GPT levels of post-freeze seminal plasma (29.87 and 8.55 μ mole) were significantly higher than that of pre-freeze levels (19.85 and 4.67 μ mole resp.). The effect of season was also found to be highly significant. GPT level was significantly and positively correlated with AKP (0.893) and freezability (0.899).

The mean sperm motility for all stages of freezing was significantly depressed by adding acetylcholine and histamine than by hyalase and non-additive groups. All three levels of additives resulted in significantly lower sperm motility. High concentration of additive depressed motility significantly than low concentration. The effect of dilutors and periods (stages of freezing) on motility was highly significant. EYCG and LYG diluents showed significantly higher motility than TFYG diluent.

The overall fertility rate with 1155 frozen semen inseminations was 46.41 %. Bulls with good freezability showed fertility rate of 50.50 %, while 40.00 % fertility rate was obtained for bulls with poor freezability. The effect of additives on fertility was found to be non-significant. Among additives, histamine revealed lowest fertility (35.13 %), acetylcholine showed highest fertility (51.96 %) and the hyalase being intermediate (48.17 %). The use of additives distinctly improved fertility (from 41.53 to 50.07 %). This improvement in fertility rate with semen additives appeared to be marked in bulls with poor freezability (47.89 Vs 52.03 %). Thus, poor freezability bulls had a decided advantage of semen additives for increasing their rate of fertility.

IJAR 7: 2: 100-101, 1986

Superovulation, Non-Surgical Recovery And Embryo Transfer In The Water Buffalo (*Bubalus bubalis*)

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Embryo transfer in dairy cattle is a tool to produce large number of high quality seed stock and to multiply the most desirable genotype. The present study was an attempt to adopt the standard superovulatory regimen, non-surgical recovery and embryo transfer technique of cattle to the water buffaloes. The experimental animals comprised of high yielding six Surti breed and six Mehsana breed buffaloes to serve as potential donors and five Surti breed buffaloes as recipients. The study was undertaken during the period from February to May, 1986 (Summer).

The synchronization of estrus was accomplished by administering Lutalyse (25 mg, I/M) a synthetic prostaglandin $F_{2\alpha}$, twice at an interval of 10 days. The mean time interval from the second PG injection to the occurrence of external signs of heat in Surti and Mehsana breed buffaloes was 70 ± 3.57 hrs.

The donors were superovulated during the mid-luteal phase by injecting 50 mg of FSH-P in nine divided doses. In Surti buffaloes, the ovulatory response following the gonadotrophin treatment ranged from 1 to 8 with the ovulation rate being 70%, whereas in Mehsana buffaloes the response ranged from 1 to 6 with the ovulation rate being 54%. The mean numbers of corpora lutea and anovulatory follicles ($>$ 10 mm) were 4.00 ± 1.13 and 1.67 ± 0.33 in Surti donors and 3.17 ± 0.91 and 2.67 ± 0.49 in Mehsana donors, respectively, the difference being non significant between the two breeds.

All the donor buffaloes were flushed between 112-116 hrs after the first insemination which was done 56 hrs after the administration of first PG injection. The flushing media used was modified phosphate buffered saline, whereas the flushing equipment used were either a 3-day stainless steel catheter with an extensible head and an inflatable cuff or a 2-way Lampeter catheter or a 2-way siliconized Rusch catheter. In all 13 embryos in various preimplantation stages were recovered from 4 Surti and 4 Mehsana buffaloes, giving a mean of 1.6 embryos per donor. The physiological age of the water buffalo embryos was 48-72 hrs ahead of chronological age when compared to cattle embryos.

The mean diameter of the morula with zona pellucida was found to be 155.90 μm, while that of late morula measured 148.50 μm. The zones of both the morula

and late morula measured out to be 22.25 μm.

For determining the effect of super-ovulation and non-surgical recovery of embryos on milk yield, the difference in average milk yields pre and post trials was tested. The paired 't' test revealed non-significant difference in the milk yield.

The viable embryos recovered were transferred non-surgically, one each to the 4 Surti recipients on day 5 of the estrous cycle. Pregnancy diagnosis was established by Enzyme-Immuno-Assay Kit (Ovucheck, International Embryos, UK) 20 days subsequent to the transfer. Out of the 4 recipients examined for milk progesterone levels, two were found negative, one doubtful and one positive on comparing the results with the standards for estrus and pregnancy.

IJAR 7: 2: 102-103, 1986

A Study On Repeat Breeding Conditions In Crossbred (K×J & K×HF) Cattle With Special Reference To Cervical Mucus

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The present study was undertaken with a view to elucidate some of the etiological factors involved in repeat breeding conditions in cross-bred cattle with reference to cervical mucus content and changes in this physical properties and suggest therapeutic measures.

Detailed gynaecological examinations of a total of 38 repeat breeding cross-bred animals showed 23.68% genital abnormalities with maximum 10.52% disorders were found to be of cervix.

Inter-oestrual lengths of 403 oestrous cycles in 38 (K×J& K×HF) heifers and cows were studied for the effect of breed, season, months and parity. Abnormal cycle lengths were observed in 58.33% of heifers and 52.43% of cows. A total of 55 cycles in 29 normally breeding cows and heifers were also analysed. Abnormal cycle lengths were found to be observed in 49.09% of the oestruses studied.

Out of 403 oestruses analysed to study the effect of months and seasons on the occurrence of oestruses, 156 (38.71%) were exhibited during cold months, 125 (31.02%) during hot months and 122 oestruses (30.27%) during wet months. Maximum oestrus periods (12.16%) were exhibited in the month of January and minimum (5.12%) in the month of September.

A total of 67 oestrus periods of 28 repeating animals were observed to study the intensity of oestrus. Out of these, 20.89% expressed weak oestrus behaviour, whereas in 29.85% of oestrus, behaviour was very good. However, 49.26% oestruses showed ptimum expression of heat. Uterine tone on rectal palpation was scored as +, ++, +++. Out of 67 oestrus periods studied 43.29% oestruses scored +++ uterine tone, while 17.91% oestruses scored + uterine tone.

Occurrence of Graafian follicles was observed to be equal (50%) in both the ovaries.

Ovulatory disturbances were studied in 37 repeating cross-bred cows and heifers. The extent of delayed and anovulatory conditions in heifers were found to be 29.41% and 35.29%, whereas in cows it was 52.50% and 22.50%, respectively. The mid-cycle oestrus was observed in 23.52% cycles of heifers and 5% cycles of cows.

The physico-chemical analysis of cervical mucus in 7 normal and 21 repeat breeding animals was done. The values for hydrogen ion concentration was 7.07 ± 0.10, & 7.50 ± 0.13, spinbarkeit 8.57 ± 1.14 & 8.24 ± 0.48 cm, conductivity 9.35 ± 0.53 & 10.59 ± 0.44 Ohms, sodium content 361.0 ± 56.47 & 293.27 ± 26.88 mg/100 ml, potassium content 27.51 ± 3.60 & 33.32 ± 3.47 mg/100 ml were observed for fertile and repeating animals, respectively. The differences in the values were non-significant. However, sodium and potassium contents during early and mid heat periods showed significant ($P<0.05$) differences.

Out of 21 mucus samples collected, only 54.17% samples were clean, transparent in repeating animals as against 71.43% samples in fertile group of animals.

There was no significant difference in the occurrence of typical fern pattern behaviour in cervical mucus of repeating and fertile group of animals, however 4.76% of the samples showed no patterns at-all in repeating animals.

A total of 14 samples from repeating and 7 samples from fertile group of animals were subjected to sperm cervical mucus invasion test. Progressively motile spermatozoa were observed in 50% and 85.71% of the samples collected from these groups, respectively.

A total of 21 and 7 cervical mucus samples from repeating and fertile group of animals were analysed for the concentration of trace elements using Atomic Absorption Spectrophotometer. The values for iron, zinc, copper and manganese in repeating animals were 23.98 ± 4.71, 33.82 ± 11.14, 0.94 ± 0.23 and 0.62 ± 0.17 ppm, whereas in fertile group only iron and zinc were detected as 36.93 ± 7.02 and 52.63 ± 11.16 ppm respectively, while that of copper and manganese were in traces.

Cervico-vaginal mucus samples from 24 repeating and 7 normally breeding animals were investigated for primary isolation of bacteria. Out of them 87.5 and 25.0% of the samples were found positive, respectively. The antibiotic sensitivity test revealed wide variation in the sensitivity pattern. Majority of the samples (95.65%) were resistant to penicillin and C. sulpha and sensitive to gentamycin (65.22%) and neomycin (52.17%).

After diagnosing the etiological factors repeat breeders were treated accordingly. For infectious causes 21 animals were treated with Gentamycin (13) and Dicrysticin-S (8) as per their sensitivity testing. The pregnancy rate was 38.09%. For the treatment of ovulatory disturbances, hormonal preparations and triple sulphate mixture were used resulting into (5) 83.33% pregnancy with Chorulon followed by triple sulphate (6) 66.66% and progesterone (3) 33.33%.

Thus, it is concluded that if hormonal treatment or triple sulph suppliment is given with a proper diagnosis of the functional disorders or trace element deficiencies, this will bring down the repeat breeding problems to a considerable extent and would be of great help in settling the animals.

IJAR 7: 2: 104-105, 1986

Gross Biometric And Histological Studies Of Prepuce And Penis With Reference To The Distribution Of Preputial Glands And Release Of Penile Adhesions In The Surti Buffalo

M.V.Sc. Thesis 1986
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The gross and biometric study was made on the genital organs of 25 male Surti buffalo calves of 4-11 months in age and six adult Surti buffalo (*Bubalus bubalis*) of above 4 years in age to measure the length and diameter of penis, the length of the glans, the depth (length) of the preputial cavity and the diameter of the preputial orifice.

Microscopic observations on H & E stained, Hart's elastic stained, Alcian blue (pH 2.5) — PAS stained and picric acid stained cryostat and paraffin sections, were made on 6 buffalo calves of 4-11 months of age, 6 buffalo calves of 15-20 months of age and on 6 adult buffalo bulls above 4 years of age. The capacity of prepuce and its shape, were studied by contrast radiographs with aqueous barium sulphate on 6 live buffalo calves (4-11 months), 6 organs from cadevers of buffalo calves (4-11 months) and 6 organs from the cadevers of adult Surti buffalo bulls above 4 years in age.

The study on penile adhesions with prepuce and its release with reference to age and body weight, was made on fifty-one live animals to observe complete adhesions (17 animals), partial adhesions (21 animals) and complete release of adhesions (13 animals). Seventeen young buffalo calves of 300 days (10 months) to 500 days (16 months and 20 days) were tranquilized with intravenous injection of 'Largactil'R at the rate of 50 mg/100 kg body weight to relax the prepuce and then the prepuce searched by inserting the fingure into it. Similarly twenty-one animals of 450 days (15 months) to 600 days (20 months) were made to mount on treasers and then their prepuce was searched manually after tranquilization with intravenous injection of 'Largactil' as described earlier. Thirteen Surti male animals of 600 days (20 months) to 800 days (26 months and 20 days) were also made to mount on teaser to see the protrusion of penis and subsequently their prepuce was searched manually as described earlier.

The average length of the penis in buffalo calves (4-11 months) was found to be 48.16 ± 0.66 cm and that of the adult bulls (above 4 years) was found to be 80.17 ± 2.83 cm, while the penile diameter in front of the sigmoid flexor was 0.76 ± 0.03 cm in the calf and 1.85 ± 0.15 cm in the adult. The length of the

glans penis was observed to be 6.16±0.12 cm in calf and 9.82±0.44 cm in the adult. The depth (length) of the prepuce was found to be 8.55±0.29 cm and 29.17± 2.24 cm in the calf and the adult respectively. The diameter of the preputial orifice was 1.18±0.04 cm in the calf and 2.37±0.2 cm in adult. The capacity of the preputial sac was measured to be 15-20 ml in the calf and 50 ml in the adult. The length of the hairy, pigmented, folded, inner preputial skin was found to be 3.26±0.07 cm in the calf and 7.58±0.36 cm in the adult. Contrast radiographs revealed the shape of the prepuce to be conical in the calf and cylindrical in the adult.

The inner, hairy, pigmented and folded skin possessed well developed sebaceous glands associated with hair. The coiled tubular glands as seen on the external preputial skin, extended upto preputial orifice. There was no evidence of any type of glands beyond the hairy, pigmented skin inside the preputial cavity. The skin reflected over the penis and skin over the glans penis did not possess any type of glands. The elastic fibres were present in papillary layer of the dermis and were marked in the pigmented hairy inner skin of the prepuce as well as on the glans penis. The other parts of the preputial skin had less amount of elastic fibres. Solitary lymphnodules were observed in the skin reflected over the penis but they were not observed else where. The stratum corneum, the collagen fibres in dermis, the subcutaneous striated preputial muscle bundles and the musculosa of blood vessels, were strongly PAS positive (pink) while the surface epithelium and the epithelium of the glands took magenta colour. The patches of alcianophilic material were seen between collagen fibres in the dermis. The lower layer of the epidermis in hairy folded, pigmented region of the prepuce, possessed melanin granules.

Adhesions between penis and prepuce were complete in the buffalo calves of 300 to 500 days which weighed from 100 kg to 190 kg. In these animals the microscopic observations showed a solid epithelial ring between the glans and the prepuce (balanopreputial fold) which gave out branching secondary folds radiating outside in prepuce while there was a dense connective band (frenulum) covered by stratified squamous epithelium from the ventral aspect of the urethral opening on glans to ventral preputial skin. The adhesions were partial in the buffalo calves of 450 to 600 days having body weight between 175 and 245 kg. They could not protrude the penis on mounting and search of preputial cavity revealed a frenular adhesion. The microscopic examination of sagital sections showed a band of dense connective tissue as well as some amount of epithelial fold between the glans and the prepuce as described in the earlier group. The animals of 590 to 854 days having the body weight between 245 and 290 kg protruded the penis on mounting and the manual search of prepuce did not reveal any frenular adhesion. Microscopic examination did not show any trace of the frenulum.

All the forty-four animals were actually weighed on the weigh-bridge and their weight was also calculating by Hall's (1971) formula in the horse, using heart girth and body length. The average difference between the two methods was found to be 1.51±0.7 kg with C.V.% 30.5. This difference was significant at $P<0.05$ when paired 't' test was performed.

IJAR 7: 2: 106-111, 1986

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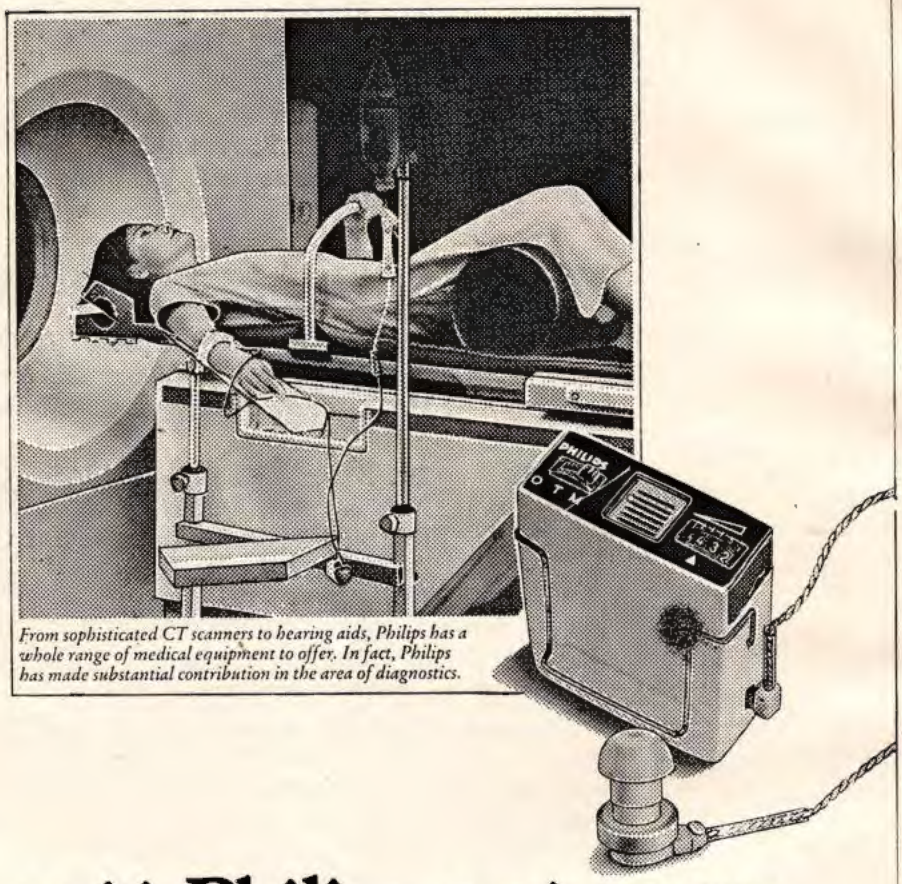

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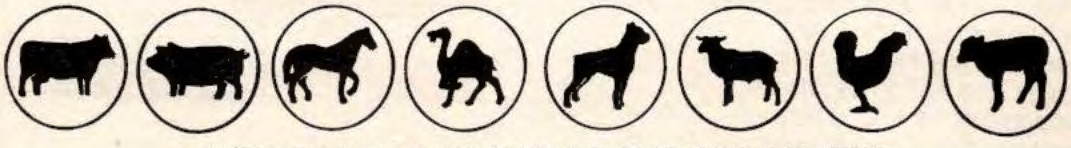

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