"LEVEL OF ELECTROLYTES IN OESTRUAL MUCUS AND THEIR CORRELATION WITH THE PENETRATION RATE OF CHILLED AND FROZEN SEMEN OF FRIESIAN AND JERSEY BULLS"

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NDRA AGRICULTURAL UNIVERSITY, BIHAR

PUSA (Samastipur)

1980

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(ABSTRACT)

THESIS
SUBMITTED TO THE
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR
PUSA (SAMASTIPUR)

By

YOGENDRA KUMAR

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF
Master of Veterinary Science (Veterinary Gynaecology
and Obstetrics)

1980.

THESIS ABSTRACT

The present study was conducted on 67 cows which were brought to the out-door clinics of Gynaecology Department of Bihar Veterinary College, Patna. Gynaecological checkups were made on all animals by means of visual examination and rectal palpation of genital organs. Oedematous swelling of vulva, congestion of vaginal mucus membrane and condition of the cervical os were noted. Uterine tones were classified on the basis of intensity of heat in first, second and third degree. Oestrual mucus samples were separately collected during different phases of oestrus. Arborisation pattern of mucus was noted. PH of mucus samples were calculated by means of 'Systronic' Digital PH meter.

Chilled and frozen semen samples were brought to the laboratory from semen and Frozen Semen Bank, Patna respectively. Motility of sperm were examined and only the semen samples of good quality were used for penetration test. Penetration rate of chilled and frozen semen in oestrual mucus collected at Ist, 2nd and 3rd degree uterine tones were made by means of sprojt OL Syringe, specially made slide and plain capillary tubes.

Level of sodium and potassium in oestrual mucus were estimated by flame photometer.

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PUSA (Samastipur)

1980

DEPARTMENT OF
VETERINARY OBSTETRICS AND GYNAECOLOGY
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR

C E R T I F I C A T E

This is to certify that the thesis entitled "Level of Electrolytes in Oestrual Mucus and their Correlation with the Penetration rate of Chilled and Frozen Semen of Friesian and Jersey Bulls" submitted in partial fulfilment of the requirement for the Degree of Master of Veterinary Science (Veterinary Gynaecology) of the Faculty of Post-graduate studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by Dr. Yogendra Kumar under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been dully acknowledged.

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We the undersigned, members of the Advisory Committee of Dr. Yogendra Kumar a candidate for the Degree of Master of Veterinary Science with major in Veterinary Obstetrics and Gynaecology have gone through the manuscript of the thesis and agree that the thesis entitled "Level of Electrolytes in Oestrual Mucus and their Correlation with the Penetration rate of Chilled and Frozen Semen of Friesian and Jersey Bulls" may be submitted by Dr. Yogendra Kumar in partial fulfilment of the requirements for the Degree.

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C E R T I F I C A T E

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A C K N O W L E D G E M E N T S

ACKNOWLEDGEMENTS

I express my profound sense of gratitude and reverence to Dr.S.N. Sinha, M.Sc.(Vet.), P.G., F.R.V.C.S. (Sweden), Professor and Head of the Department of Veterinary Obstetrics and Gynaecology, Bihar Veterinary College, Patna for his precious guidance, invaluable suggestions and stimulating counsel. The forbearance with which he supervised the work and trust he conferred on me was always a source of inspiration in the midst of difficulties.

I am also grateful to the Vice-Chancellor, Rajendra Agricultural University, Bihar and Drs. H.R.Mishra, Dean-cum-Principal, Ranchi Veterinary College, Ranchi, R.N.Singh and S.A. Ahmad, the respective Principals, Bihar Veterinary College, Patna and A. Ahmad, Director Resident Instruction-cum-Dean, Post-graduate, Rajendra Agricultural University, Bihar for providing teaching and research assistantship and for extending laboratory and library facilities during the period of present study.

I would like to extend my cordial thanks and indebtness to Drs. Md.H.Akhtar, Asstt.Prof. Gynaecology, H.N. Thakur, Assoc. Prof. Pathology, A.K. Barnwal, Assoc. Prof. Anatomy, M.K. Singh, Assoc. Prof., S.P. Sinha, Asstt.Prof. Pharmacology, B.P.Singh, R.P. Gupta, Asstt.Prof. Medicine, C.B. Prasad, Assoc.Prof. Microbiology, R.B. Singh, Assoc.Prof. Gynaecology and M.K. Roy, Asstt. Prof. Anatomy of Bihar Veterinary College, Patna for their skilful help, good advice, profitable discussions and co-operation during the present study.

I express my heartiest thanks to Drs. B.N. Verma, Project Officer, Frozen Semen Bank, Patna and Indrabhan Prasad, A.I.O., Semen Bank Patna for providing chilled and Frozen Semen of Jersey and Friesian bulls for the present work.

My thanks are due to Dr. Arbind Kumar, T.V.O.,Nimdih, Singhbhum for his kind help during statistical analysis of the research work.

My most cordial thanks are also due to Drs. J.N.Singh, B.P.Singh, I.D. Singh, M.P. Singh, R.P. Verma (Post-graduate students) and Karan Bohra, Damodar Sedai, Shiv Kumar Sharma (under-graduate students) of Ranchi and Bihar Veterinary College, Patna for their friendly co-operation and constant encouragement during to course of the thesis work.

Last, but not the least, I shall be failing in my duty if I do not express my whole hearted appreciation to my old parents, and other members of the family for their invaluable sacrifice and long patience which in turn proved to be a deep rooted source of encouragement for completion of this study.

Kumar.

(Y. Kumar)

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INTRODUCTION

Cervical mucus secretion and tonicity of the uterus are the most important signs of oestrus (Roberts 1971). Examination of these signs is done routinely in gynaecological check-up before the cases are deemed fit for insemination. The cervical mucus secretion becomes evident in the follicular phase of the oestrus cycle which tends to become copious, thin, watery and stringy as the phase advances to culminate in the occurrence of oestrum. The tonicity of the uterus also follows the same pattern of changes and is maximum when the cattle is in standing heat. Thus, there exists a great correlation between the physiochemical properties of oestrual mucus and the tonicity of the uterus (Salisbury and VanDemark, 1961). After insemination millions of live and progressively motile spermatozoa penetrate the oestrual mucus as they move towards the cervix and uterus to reach the fallopian tube which is the ultimate site of fertilization. Thus the sperm are exposed to the biochemical environment of oestrual mucus for quite a long period. The physiochemical properties and the biochemical environment of the oestrual mucus, therefore, markedly influence the livability and migration of sperm. The quality and the biochemical nature of the oestrual secretion is very important for the penetration of viable sperm.

Very few elucidative attempts have been made to study the physiochemical character of oestrual mucus collected during different degrees of uterine tone and the penetration rate of sperm in those oestrual secretions. Akhtar (1979) studied the migration rate of spermatozoa in oestrual mucus collected in normal and diseased conditions of genital tract in cattle.

With the adoption of crossbreeding as a national policy and the extensive use of germ plasm of exotic breeds especially of Holstein Friesian and Jersey bulls well directed studies on this aspect seem to be rather imperative. Exploration of this aspect of sexual physiology would help in assessing the quality of oestrual mucus secretion and also the quality of the semen samples. Informations thus gained would help in augmenting crossbreeding programme and improving pregnancy results for more economic gains to the livestock owners. With this aim in view the present study was undertaken to study the following :-

1. Physiochemical characters of oestrual mucus collected during different degrees of uterine tone.
2. Electrolyte concentration of oestrual mucus secretion.
3. Estimation of pH of oestrual mucus secretion.
4. Pentration rate of liquid and frozen semen of Holstein Friesian and Jersey bulls in oestrual mucus secretion in relation to electrolyte concentration and different degrees of uterine tone.
5. Arborisation pattern of oestrual mucus secretion.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Consistency of the oestural mucus.

Oestrual mucus is the mixture of clean, transparent fluids of luminal, uterine and cervical secretions. As during oestrus period it flows out from external genitalia through cervix so it is commonly said to be cervical mucus. The most important sign of oestrus is the out flow of the mucus from external genitalia of cow. The degree of viscosity (thin, medium and thick) represents the duration of oestrus period.

Roark and Herman (1950) studied cervico vaginal oestrual mucus of 100 cows and reported that the diagnosis of early and late oestrus can be made by the character and consistency of the mucus secretion.

Scott Blair (1956) found that the physical and chemical properties of oestrual mucus represented the phases of oestrum and oestous cycle.

Bacic (1962) examined 575 oestrual mucus samples collected from 523 cows and reported 79 to 83 per cent conception rate in clear mucus while it was reduced by 11 to 53 per cent according to the quantity of pus present in the sample. He further found that there existed between viscosity and elastisity.

Zust (1966) recommended pH viscosity of oestrual vaginal mucus as a suitable method for detecting oestrum

in cows and reported low viscosity of vaginal mucus before ovulation in 23 Black Pied Low Land Cows.

Pattabiraman et al. (1967) observed the viscosity of oestrual mucus in 58 healthy breeding cows, the number of mucus samples collected in early, mid and late heat were 25, 25 and eight respectively. The viscosity of the oestrual mucus was graded as thin, medium and thick depending upon the rate of flow.

pH of the oestrual mucus.

Smith and Asdell (1941) found alkaline pH of cervico vaginal mucus during oestrus and metestrus in 30 healthy cows. The pH varied in the range of 7.0 to 8.9 with an average of 7.9. The cervical mucus was more acidic than vaginal mucus.

Choudhari and Prasad (1954) examined the pH of oestrual vaginal mucus of 135 normal Tharparkar cows ranging between 6.0 to 8.5 in 41.4 per cent and 7.0 in 26.6 per cent of the mucus samples examined. The pH was not affected by age or stage of lactation.

Olds and VanDemark (1957) observed higher pH range of vaginal and uterine secretions during oestrum.

Shalash (1958) found mean pH to be 7.3 in vaginal secretion of 58 healthy she-buffaloes.

Hartwig (1959) reported pH range of 6.12 to 8.50 with an average of 7.24 of 269 bovine oestrual mucus samples.

Taubrich (1959) examined oestrual mucus samples 179 and 117 times from cervical and vaginal portion in 96 cows for pH and found it to be 7.7 to 7.9 in cervical region. The pH in the anterior portion of vagina, however, was always (0.2 to 0.4) higher and was not lower than 0.5 during oestrum.

Ahuja et al. (1961) studied oestrual mucus samples collected from 114 cows in 107 oestrous cycles and reported the average pH to be 7.18 ± 0.08, 7.33 ± 0.04 in cows and six heifers of Hariana breed.

Valdivia and Vallenas (1961-62) found the pH to be alkaline in the oestrual cervical mucus of 19 healthy cows.

Bacic (1962) observed the pH to be varying from 6.9 to 9.0 with a mean of 7.6 in 578 oestrual vaginal mucus samples of 523 cows.

Gupta (1962) observed the pH of oestrual cervical mucus to be 7.25 to 8.0 with an average of 7.91. On further extending his study on 40 more samples of oestrual cervical mucus of cows he found the mean pH to be 7.6 and concluded that pH varied in successive samples from the same cows.

Gupta et al. (1962) examined 40 cervical and 21 uterine fluid samples collected from 38 dairy cows during

oestrus and reported average pH of cervical and uterine fluid to be 7.81 and 7.91 respectively.

Hukeri (1965) studied 346 samples of oestrual cervical mucus of 103 Tharparkar cows and reported average pH to be 8.35 ± 0.04.

Zust (1966) examined vaginal mucus samples collected during oestrum from 23 Black Pied Low Land Cows. The oestrous cycle of cows was normal. He reported the pH of vaginal mucus to be 6.4 to 8.0.

Pattabiraman et al. (1967) reported pH of oestrual cervical mucus to be 7.0 to 9.0 with an average of 8.03 ± 1.22.

Kouser (1969) observed the pH value of uterine, cervical and vaginal fluids collected during oestrum from 20 normal and 125 repeat breeding cows of Tharparkar breed and reported average pH value as below :-

	Average pH in normal breeding cows	Average pH in repeat breeding cows
(a) Uterine secretion	7.21±3.2	7.10±0.21
(b) Cervical secretion	7.41±0.21	
(c) Vaginal secretion	6.21±0.13	

Akhtar (1979) examined 20 normal, 10 repeat breeders and 10 cows affected with cervicitis. The mean pH of cervical mucus during oestrus in normal, repeat breeders

and cases of cervicitis was found to be 7.885 ± 0.057, 7.730 ± 0.153 and 7.390 ± 0.090 respectively.

Luktuke (1974) recorded average pH of vaginal mucus in Hariana Heifers and cows to be 7.18 ± 0.08 and 7.33 ± 0.04 respectively.

Reddy (1974) reported the mean pH of bovine cervical oestrual mucus to be 7.32 whereas Wani et al. (1979) observed the mean pH of bovine cervical oestrual mucus to be 6.69 ± 0.24.

Arborisation pattern of oestrual mucus.

Higaki and Awai (1953) reported frizzy hair like cervical mucus pattern after 35 days of insemination of cows. They found 95 per cent accuracy in pregnancy diagnosis by the test.

Bone (1954) examined the physical properties of cervico vaginal mucus of 183 cows during oestrum. He reported that the arborisation pattern was related to progesterone activity. He further commented that it was not a suitable method to determine pregnancy and reproductive activity.

Pozolora (1955) described the technique for collection of cervical mucus. After microscopic examination of the dried mucus he classified it according to the presence or absence of the crystals and specific fern-like structure formed by the crystals. The procedure was

subsequently adopted to diagnose the various stages of the oestrous cycle.

Coluzzi and Battistacci (1956) reported that crystallisation test of cervical mucus was not a reliable method for diagnosing preganancies.

McDonald and Raesida (1956) examined cervical mucus collected from pregnant, castrated and hysterectomized ewes as well as from ewes which were administered oestrogen progesterone and gonadotropin. Fern-like patterns were present in association with oestrus and it was concluded that the cervical mucus smear was highly sensitive to oestrogen and progesterone. The technique was found suitable to determine the quantitative production of ovarian hormones in ewes.

Horvath (1960) studied the crystallisation pattern of cervical mucus of 69 mares, 30 cows and 18 bitches. The experiment was conducted to know the ovarian function, phases of oestrous cycle, nymphomanic condition in cystic ovaries in mares and cows, pregnancy diagnosis in mare and right time for mating in bitches.

Howes et al. (1960) diagnosed 81 per cent ovulation on the basis of cervical mucus crystallisation test in cattle and reported the test to be the best method for detection of ovulation in bovine.

Bane and Rajakoski (1961) reported more pronounced arborisation of fern-like pattern of dried oestrual cervical mucus smears of cows.

Luktuke and Subramaniam (1961) found three types of crystallisation pattern in bovine cervical mucus. Classification was made on the basis of oestrogen level of the mucus. Type-A was associated with the highest oestrogen level, B was intermediate and C appeared with lower level of cyclic oestrogen. They did not find crystallisation in 91 per cent of pregnant cows. They recommended the method suitable for the detection of the phases of estrous cycle but unsuitable for pregnancy diagnosis because they observed no crystallisation when progesterone level was predominant. The crystallisation pattern of vaginal mucus was mostly of B type and was of no diagnostic values.

Sysoev (1961) reported arborisation pattern of cervical mucus in some of the five and half-month pregnant cows. He observed arborisation patterns in all the specimens at one or two months before calving.

Gamcik and Sevcik (1962) examined the cervical mucus samples of 147 cows during the year 1959-60. They observed first sign of arborisation before two-three days before oestrus which was more pronounced until second half of the oestrus and about the time of ovulation. Arborisation was also noted in cystic ovaries. A typical arborisation was reported in the first two months and at the end of pregnancy. Pregnancy was correctly diagnosed by the arborisation pattern in smears from 73.3 per cent of the cows in the first month, 75.1 per cent in second month and

78.2 per cent in the third month. The arborisation test was reported suitable for diagnosing the phases of oestrous cycles and as a supporting test to diagnose pregnancy.

Abusineina (1962) examined the arborisation patterns of cervico vaginal mucus of four pregnant cows. After injecting one gm of stilboestrol and 40 mg stilboestrol dipropionate daily for 22 days in a non-pregnant cow, the mucus samples were collected daily for the arborisation test. The cervical mucus was also collected from the uteri of 100 cows slaughtered at various stages of pregnancy. Three types of crystallisation were observed according to whether the mucus was translucent, acellular, elastic and easily obtainable from the cervix. Type-A were semi-clear elastic and easily obtainable. Type-B were opaque, cellular and difficult to aspirate. Type-A were observed on the day nearest to ovulation and in estrogen treated cows. Type-B occurred before and after four days of ovulation.

Senze and Zebracki (1962) tested the oestrual cervical mucus smears of 100 cows the object of which was to determine indirectly the oestrogen concentration in cervical mucus at the time of estrus. In absence of clear reaction in 44 cows they suggested for hormonal treatment for the animals. Thirty of the cows in this group conceived after intramuscular injection of 20 mg of stilboestrol. Fifty of the other 56 animals conceived after improvement in feeding and management.

Hukeri (1965) examined 185 oestrual cervical mucus smears for arborisation and reported typical (69.33%), atypical (23.23%) and no crystallisation pattern in 11 (7.34%) out of 150 samples of cervical mucus.

Prokopanova et al. (1965) studied arborisation patterns of oestrual cervical mucus in 64 cows.

Zust (1966) observed similar crystallisation patterns of vaginal mucus in all stages of oestrus and found the method unsuitable for diagnosis of oestrum.

Changes in the genital organs during oestrum.

Perkins et al. (1950), Hall et al. (1959a), Salisbury and VanDemark (1961) reported average time of ovulation to be 26.33 hours from the onset of oestrum.

Laing (1951) reported that there was correlation between the time of ovulation and the duration of oestrum in forty cows.

Ivankov (1956) confirmed 59.4 per cent ovulation in left ovaries of 32 cows and heifers as well as 77.7 per cent from right ovaries of nine heifers after slaughter.

Varenika (1957), Ahuja et al.(1961) and Hukeri (1965) reported 60.90 per cent ovulations from right ovaries. The occurrence of ovulatory heat was observed to be 70.62 per cent.

Perkins et al. (1959) found no correlation between the time of ovulation and duration of oestrum.

Howes et al. (1960) examined the smears of the oestrual mucus of Bos taurus and Bos indicus (Brahaman) heifers for the detection of ovulation. The findings of the study of arborisation patterns of smears were later confirmed by rectal palpation on the basis of the presence of ovarian corpus luteum.

Kiesel and Grimm (1961) reported greater number of ovulations with the rise of environmental temperature in 7,000 cows.

Glod (1961b) observed 29.87 hours to be the average ovulation time from the onset of oestrus in 69 Polish red (PR) and 54 Polish Black Pied Low Land (BPL) cows. He recorded 47.67 per cent, 52.33 per cent ovulations from the left and right ovaries respectively.

Kidder et al.(1962), Van Rens Burg and Devos (1962) reported 56.5 per cent ovulations on the basis of rectal palpation in the ovaries of 564 cows. Kidder observed 1.92 per cent multiple ovulations in the haphazard manner. Hahn and Sberanardori (1962) found significant correlation between the size of ovaries and (a) contractibility of the uterus (b) hyperaemia of the vestibular mucosa. They also correlated the uterine contractibility with the size of the ovarian follicles in heifers. The intensity of the sign of oestrum and the uterine contractibility also decreased

after ovulation in cows and heifers.

Hukeri (1965) examined 149 Tharparkar cows and reported 60.90 per cent, 39 per cent ovulations from right and left ovaries respectively. He further reported highly oedematous swelling of vulva, hyperaemia of vaginal mucus membrane, highly hyperaemic and dilated condition of the outer part of the cervix, very hard, tonus, turgid uterus in pronounced oestrum. In normal oestrum, vulva was oedamatus, vaginal mucus membrane was hyperaemic, outer cervix hyperaemic, dilated accompanied by turgid and easily palpable uterus. In weak heat, the vulva was some time oedematous, vaginal mucus membrane hyperaemic and partially dilated outer part of the cervix.

Penetration rate of sperm in oestrual mucus.

Herman and Horton (1948) observed the highest penetration rate of spermatozoa in oestrual cervical mucus during the first six to ten hours of oestrus.

Roark and Herman (1950) found the maximum penetration rate of spermatozoa in oestrual cervical mucus, collected between 10 to 13th hours from the onset of the oestrum to be 2.81 milimeter/minute and the range was between 0 to 6 milimeter/minute.

Berrang (1951) studied the penetration power of spermatozoa by using capillary tubes filled with oestrual

mucus. Intensity of sperm motility was found to be a decisive factor. Sperm concentration had no effect. Semen of equal quality from different bulls possess different power of penetration. The penetration rate was reported greater in vaginal than in the cervical mucus.

Moellar and VanDemark (1955) reported the average speed of bovine spermatozoa in oestrual mucus to be 114 micron/second with an average of 10 to 353 micron/second. Significant differences in spermatozoal speeds were found in different ejaculates.

Tassinari (1957) compared sperm penetration through mucus held vertically in a capillary tube one end of which was closed. The test was considered positive after certain time, the presence of ambient temperature and length of the tube, the presence of live spermatozoa at the top of the capillary tube. The test was reported to be 66 per cent accurate.

Roslanowski and Koefee-Johanson (1959) established a positive correlation between follicular development viability and speed of spermatozoa in cervical mucus collected at 12 hours interval during oestrum. Highly significant correlation between penetration and livability with conception rate was observed.

Guard (1960) reported a new technique to determine the penetrative capacity of spermatozoa into the oestrual

cervical mucus by using a modified haemocytometer chamber. The sperm mucus penetration rate was ascertained by counting the penetrated spermatozoa in the oestrual cervical mucus kept on the counting chamber for the purpose.

Harvey (1960) correlated the average speed of human spermatozoa with fertility index. Men with a fertility index of 50 or more were regarded to be of adequate fertility than those with index between 30 and 40 as somewhat bellow normal while the men below fertility index of 30 were said to be subfertile.

Tampion and Gibbons(1962a) found during their experiment that, spermatozoa travelling in a thread of mucus were preferentially oriented in the direction in which the thread had been drawn out.

Tampion and Gibbons(1962b) conducted 25 experiments with 10 semen samples. They recorded that the mean swimming rate of spermatozoa in oestrual mucus varied from 22.0 to 80.7 micron/second. The mucus samples used for the experiments were kept at $10^{o}C$ for sometimes. They further reported that the penetration rate of spermatozoa of six semen samples into six fresh oestrual mucus was 37.5 to 70.5 micron/second. They came to the conclusion from the above results that swimming rate of bull spermatozoa was only half of the movement of the female genital tract.

Henriet and Vervack (1963) reported that the penetration rate of spermatozoa was influenced by the properties of the oestrual mucus.

Akhtar (1979) studied the migration rate of sperm of Tharparkar bull in the cervical mucus of normal and repeat breeder cows. He found the average sperm migration rate in the cervical mucus of normal and repeat breeder cows was 36.2 ± 1.44 milimeter and 27.2 ± 2.71 milimeter/ 20 minutes respectively.

Fredricsson et al. (1977) observed the morphology of post-coital spermatozoa in the human cervical secretion and its clinical significance. They reported that spermatozoa within the cervical secretion exhibit significantly better morphology than the semen sample, indicating the presence of a barrier against abnormal spermatozoa at the level of the external os. The barrier was significantly more effective in cases where conception later occurred. Spermatozoa with abnormal head was detected to the barrier.

Katz et al. (1978) studied the character of movement of human spermatozoa in cervical mucus. The freely swimming behaviour of spermatozoa into the cervical mucus was studied with high speed cinemicrography. Flagellar beat frequency was higher in midcycle in human cervical mucus at 21°C than in native semen. The beat shape differed possessing diminished amplitude and wave length. Although spermatozoa swam straighter in the mucus, sperm swimming speed was compared in three media. The media were semen, cervical mucus and the Tyrode's solution. The progressive swimming speeds did not differ. Swimming speed and beat

frequency were linearly related in semen and in Tyrode's solution but in mucus the linearity was less certain.

Hallet and Craig (1979) reported average speed of normal spermatozoa to be 384 micron/second in 88 semen samples of 39 bulls. The investigation was done by the quasi-elastic light-scattering technique.

Sodium and potassium content in the oestrual mucus.

Gupta (1962) examined sixty one samples (40 cervical and 21 uterine secretions) collected from 24 Holstein, nine Jersey and five Brown Swiss cows from November, 1960 to July, 1961. The average volume of luminal fluids collected from the cervix and the uterus was about two to three ml. The average concentrations(in mg/100 ml) of various constituents in the cervical and uterine fluids respectively were sodium 316 and 372 and potassium 76 and 78.

Gupta et al. (1962) examined 40 cervical and 21 uterine fluids collected during oestrus period from 38 dairy cows. The average level of sodium and potassium in cervical and uterine fluids were observed to be 315.8, 371 mg and 75.6, 77.5 mg/100 ml respectively.

Guay (1966a-1966b) reported the mean sodium and potassium levels in normal oestrual cervical mucus to be 2.773 mg/gm and 0.799 mg/gm respectively.

Guay and Lamothe (1970) observed the mean level of sodium and potassium in the cervical mucus of normal cows to be 543.12 mg/100 ml and 61.63 mg/100 ml respectively.

Reddy (1974) reported the mean concentration of sodium and potassium in the cervical mucus of normal cows to be 361.82 ± 11.8 mg/100 ml and 53.83 ± 4.32 mg/100 ml respectively.

Shoaib (1975) reported the average level of sodium and potassium in the cervical mucus of normal cows to be 380.00 ± 11.11 and 55.36 ± 4.54 respectively.

Wani et al. (1979) reported the mean level of sodium and potassium in the cervical mucus of cattle was 340.50 ± 3.59 and 46.85 ± 1.12 mg/100 ml respectively.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was conducted on 67 cows belonging to different age groups and breeds. The animals were brought to the out-door clinics of Gynaecology Department, Bihar Veterinary College, Patna, for artificial insemination. Proper detection of oestrum was done on the basis of signs and symptoms. The oedematous swelling of the vulvae, congestion in the vaginal mucosa, condition of the cervix, tone, turgidity of the uterus and presence or absence of follicles and carpus luteum in either left or right ovary were observed by means of visual examination of the external genitalia and rectal palpation of the internal genital organs.

Examination of the cows for the detection of oestrum.

Animals were secured in the crate. Oedematous swelling of vulva and congestion of vaginal mucus membrane were noted by visual examination.

Taking due care and all the precautionary measures the left hand was introduced in the rectum of the cow. The cervix uteri was first felt. The uterine cornuaewere rolled back by giving traction on the ventral intercornual ligament. The uterine tone was classified on the basis of intensity as to first, second and third degree.

First degree tone was found in those cases which were brought about 24 hours after the onset of oestrus and in

which a follicle could not be palpated on either of the ovaries. The oestrual mucus discharge had also become scanty and slightly opalascent. Cervix in most of the cases seemed to lie ventral to floor of the vagina.

Second degree tone of the uterus was found in those cases which were brought very close to the beginning of oestrum. The uterine cornua had milder tone and the ovarian follicles were palpated to be very tense. Clear watery oestrual mucus was observed in most of the cases.

Third degree tone was found in these cases which were brought approximately during middle to end of oestrus. The uterine cornua were palpated to be contracted and uneven due to contraction of the circular muscle fibers of the uterus. The ovarian follicles were about 16 to 18 mm in diameter which tended to become soft. The oestrual mucus discharge was copious and stringy. Left and right ovaries were palpated by grasping the ovaries in between middle and fourth finger. The surface of the ovaries was palpated gently by means of thumb and second finger to ascertain the presence or absence of follicles.

Collection and examination of the oestrual mucus.

The hind quarter and the external genitalia were washed with distilled water. A sterilised metallic vaginal speculum was introduced into the vaginal canal. The condition

of the os of the cervix was noted. About 30 to 40 ml of oestrual mucus out flow was collected in a 100 ml capacity beaker. Only clear and transparent mucus sample was collected for the purpose of the present study. Immediately after collection the collected oestrual mucus was brought to the laboratory for the following examinations :-

pH of the oestrual mucus - The pH of the collected oestrual mucus was observed by digital pH meter. Bulbs of the electrodes were washed properly with sterilised neutral water and were then dried with filter paper. The electrode bulbs were dipped into the mucus samples to record the pH.

After estimating the pH of the mucus, the samples were then divided equally into three test tubes to carry out the under mentioned examinations.

Arborisation pattern - One drop of mucus sample was taken on a clean dry slide and was dried by putting the slide in an incubator at 37°C for 15 to 20 minutes. On the basis of crystallisation pattern the samples were classified as typical (+++), atypical (++) and no crystalisation.

Estimation of the sperm penetration rate in the oestrual mucus - Chilled Jersey and Friesian bull semen were obtained from Semen Bank, Patna and the frozen Jersey and Friesian bull semen were obtained from Frozen Semen Bank, Patna.

The semen samples both chilled and frozen were examined for motility before the determination of their penetration rate.

The motility of sperm was first checked by putting a very small drop of semen sample on a dry clean glass slide and the semen drop was focussed under low magnification (10X) of the microscope. Semen samples containing atleast 50 per cent motile sperm were selected for the study of penetration rate.

Determination of sperm penetration rate was carried out according to Akhtar (1979). Plain capillary tubes of 75 mm length having diameter of 1.1 mm to 1.2 mm with the wall thickness of 0.2 ± 0.02 mm (Proper Manufacturing Co. INC Long Island City, New York) were used for the purpose. One end of the capillary tube was fitted in the narrow corresponding translusent plastic connective tube. This connective tube was again fitted into another slightly longer connecting plastic tube. The connecting tube was fitted with the nozzle of the SPROJTE DL Syringe (DANSK LABORATORIES UDSTYRA/SRYESGADE 32200 KOSEN HAVN, N TIE (01) 392600) of 5 ml capacity. There was a metallic screw arrangement to push ahead the piston of the syringe. The screw was tightened to push the piston upto the mark 2 to 3 ml of the syringe. The open end of the plain capillary tube was dipped into the mucus sample. The screw was then loosened gradually. The mucus was pipetted in the plain

capillary tube. One end of the capillary tube was sealed with the ordinary soft wax. The tube was then fitted in a specially designed slide. Two small semicups made up of plastic were fitted above the border of the slide. A thick band of soft ordinary wax was arranged about 3 cm above the semispherical cups. One open end of the capillary tube filled with mucus sample was fitted in such a manner that the open end dipped in the cup of the slide. One to two drops of semen sample to be studied were kept in the cup. The open end of the capillary tube was in contact with the semen. A moist filter paper was kept in a petridish. The slide was then placed on the petridish. The petridish was kept in the incubator for 20 minutes at 37°C. The slide was taken out after incubation and capillary tube of the slide was focussed under 5X, and later on 10X of the microscope. The distance travelled by progressively motile spermatozoa in the oestrual mucus inside the capillary tube was measured in millimeter by means of stage micrometer. Frozen semen samples were thawed before they were put to penetration rate tests.

Estimation of sodium in the oestrual mucus samples - The mouth of the test tube containing oestrual mucus was plugged with cotton. Case number and date of mucus collection was written with glass pencil on the outer wall of the test tube for identification. Samples were kept in the refrigerator. Estimation of sodium was done with the help

of "Systronic" flame photometer, 0.1 ml of oestrual mucus was mixed with 9.9 ml of double glass distilled water to make it 1:100 dilution. For the estimation of sodium and potassium, the rate of the dilution was the same.

The flame photometer was calibrated by standard sodium solution of 110, 120, 130, 140 and 150 mEq/l. The reading in galvenometer for unknown samples diluted to 1:100 was noted and estimation was done from the standard curve.

Sodium standard solution - 5.85 gms of sodium chloride was dissolved in one liter of glass distilled water to prepare stock standard solution containing 100 mEq/l of sodium. Working standards were prepared from this stock standard by measuring out 1.1, 1.2, 1.3, 1.4, 1.5 and 1.6 ml into 100 ml capacity volumetric flasks and diluted to the mark with glass distilled water. These standards represented 110, 120, 130, 140, 150 and 160 mEq/l of sodium at 100 dilution. A calibrated curve was prepared from these working standard by plotting flame photometer galvanometer reading against mEq sodium content in mEq/l.

Preparation of potassium standards - Stock standard containing 10 milli equivalent of potassium per liter was prepared by dissolving 0.746 gms of potassium chloride in one litre of glass distilled water. Working standards equivalent to 10, 20, 30, 40 and 50 mEq/l at a dilution of 1:100 were prepared by taking 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml stock standards into 100 ml capacity

volumetric flasks. Glass distilled water was added to the measured mark of the flask to make the dilution to 100. Calibration curve was prepared from these working standard solutions as described in the estimation of sodium.

Estimation of potassium - The standard curve was prepared similarly from standard dilution rate of 10, 20, 30, 40 and 50 mEq/l. The galvanometer reading of flame photometer for unknown quantity of potassium in the oestrual mucus diluted to 1:100 was noted and the actual quantity was determined from the standard curve.

Milligram percentage of sodium and potassium obtained from milliequivalent by multiplying the results by 2.3 and 3.9 respectively (Shoaib, 1975).

RESULTS

RESULTS

Sodium level in oestrual mucus of cows collected at different degrees of uterine tone.

In the present study the average level of sodium in oestrual mucus in first, second and third degree uterine tone was recorded to be 228.90 ± 5.55, 278.40 ± 6.90 and 386.40 ± 5.25 mg/100 ml respectively (Table 1).

Table -1.

Levels of sodium in oestrual mucus in different degrees of uterine tone during oestrus.

Degree of tone	n	Range	Sodium level of oestrual mucus Mean ± S.E.	C.V.%
1st degree tone	17	200.1-276	228.90±5.55	9.99
2nd degree tone	21	230-333.5	278.40±6.90	11.350
3rd degree tone	29	319.7-4347	386.40±5.25	7.310

The level of sodium in the oestrual mucus was found to increase with the increase in the intensity of the uterine tone. The difference in the average level of sodium in the different degrees of uterine tone was found to be statistically significant at 1 per cent level (Table 2).

Table-2.

Showing the analysis of variance of sodium level in oestraal mucus at different degrees of uterine tone during oestrus.

Source of variance	d.f.	M.S.	F
Between degrees of tone	2	150800.15	190.124 ++
Within degrees of tone	64	793.165	

++ denotes significant at 1 per cent level.

The critical difference test in regard to the difference in mean sodium values between first and third degree (147.150), first and second degree (49.50) and second and third degree uterine tone (108) were found to be statistically significant at 1 per cent level (Table 3).

Table-3.

Showing the critical difference of sodium level between different degrees of uterine tone.

Comparison between groups of uterine tone	Mean sodium difference between groups of uterine tone	Critical difference	
		at 5% level	at 1% level
1st degree tone Vrs 3rd degree tone	147.50 ++	16.6	22.07
1st degree tone Vrs 2nd degree tone	49.50 ++	18.26	24.28
2nd degree tone Vrs 3rd degree tone	108.00 ++	15.92	21.17

++ denotes significant at 1 per cent level.

Level of potassium in oestrual mucus of cows collected at different degree of uterine tone.

In the present study the average level of potassium in oestrual mucus in first, second and third degree of uterine tone was found to be 70.24 ± 1.77, 63.90 ± 2.07 and 53.87 ± 2.72 mg/100 ml respectively (Table 4).

Table-4.

Showing the level of potassium in oestrual mucus in different degrees of uterine tone during oestrus.

Uterine tone	n	Range	Potassium level of oestrual mucus	
			Mean ± S.E.	C.V.%
1st degree	17	56.55-78.0	70.24±1.77	10.40
2nd degree	21	44.46-76.05	63.90±2.07	14.85
3nd degree	29	33.15-76.05	53.87±2.72	27.23

The level of potassium in oestrual mucus was observed to decrease with the decrease in the degree of the uterine tone. The difference in the average level of potassium between the different degrees of uterine tone was found to be statistically significant at 1 per cent level (Table 5).

Table-5.

Showing the analysis of variance of potassium level in oestrual mucus at different degrees of uterine tone during oestrus.

Source of variance	d.f.	M.S.	F
Between degrees of tone	2	1550.55	11.424 ++
Within degrees of tone	64	135.719	

++ significant at 1 per cent level.

The critical difference test in respect of the difference in mean values of potassium between first and third degrees (16.37), second and third degrees of uterine tone (10.03) were found to be statistically significant at

1 per cent level but the difference between first and second degree (6.34) uterine tone was found to be statistically non-significant (Table 6).

Table-6.

Showing the critical difference of potassium level between different degree of uterine tones.

Comparison between groups of uterine tone	Mean potassium level difference between groups of uterine tones	Critical difference at 5% level	Critical difference at 1% level
1st degree tone Vrs 3rd degree tone	16.37 ++	6.84	9.09
1st degree tone Vrs 2nd degree tone	6.34 N.S.	7.94	10.56
2nd degree tone Vrs 3rd degree tone	10.03 ++	6.56	8.72

++ significant at 1 per cent level.

N.S. denotes non significant.

Penetration rate of liquid semen of Friesian bull into the oestrual mucus of cows at different degrees of uterine tone.

In the present study the mean penetration rate of liquid semen of Friesian bull in the oestrual mucus collected at first, second and third degrees of uterine tone was found to be 37.91 $\pm$ 1.46, 49.88 $\pm$ 1.44 and 55.67 $\pm$ 1.12 milimeter per 20 minutes respectively (Table 7).

FIG-1

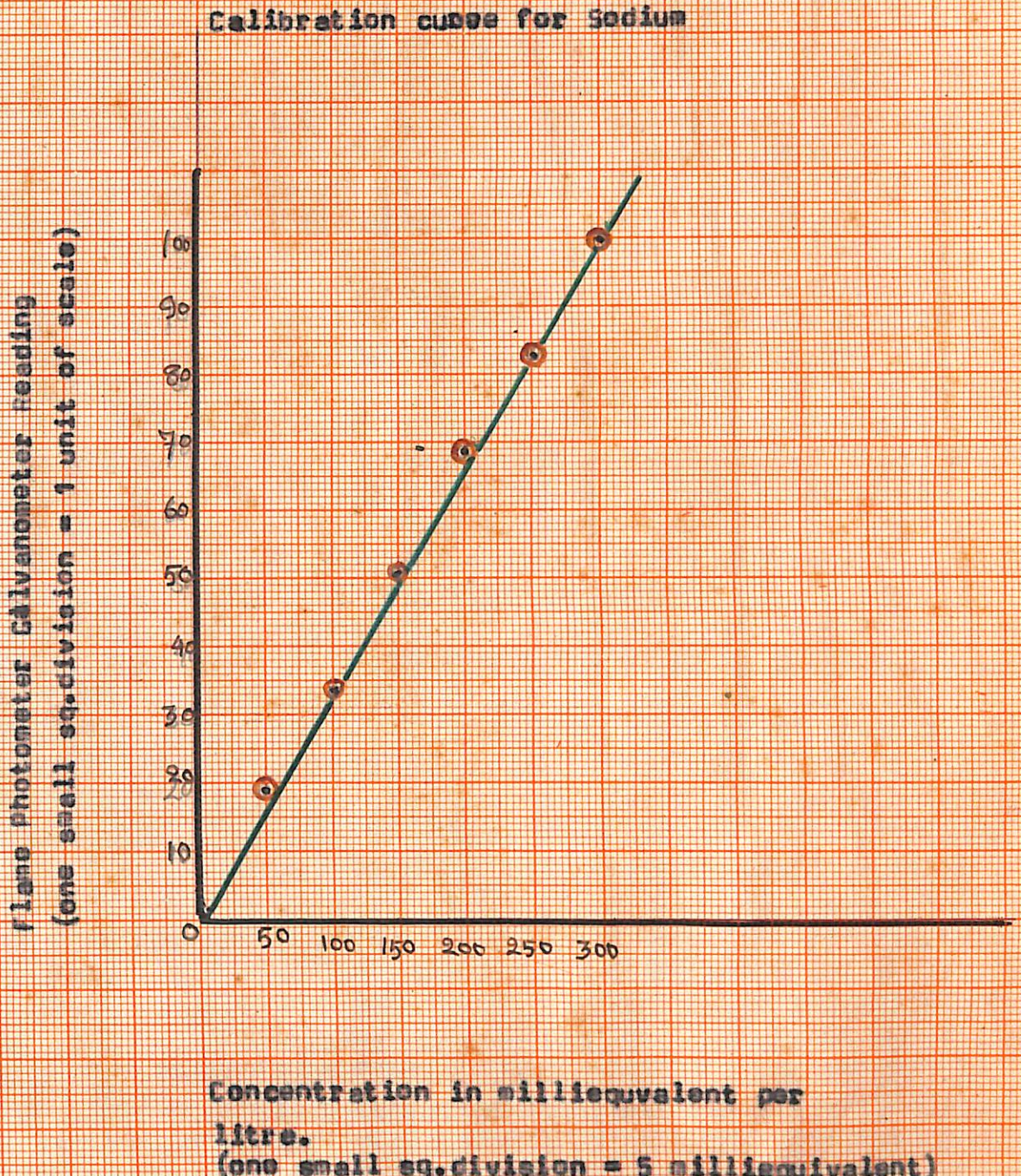

Calibration curve for Sodium
Flame photometer Galvanometer reading
(one small eq.division = 1 unit of scale)
100
90
80
70
60
50
40
30
20
10
0
50
100
150
200
250
300
Concentration in milliequvalent per litre.
(one small eq.division = 5 milliequivalent)

FIG-2

Calibration curve for Potassium

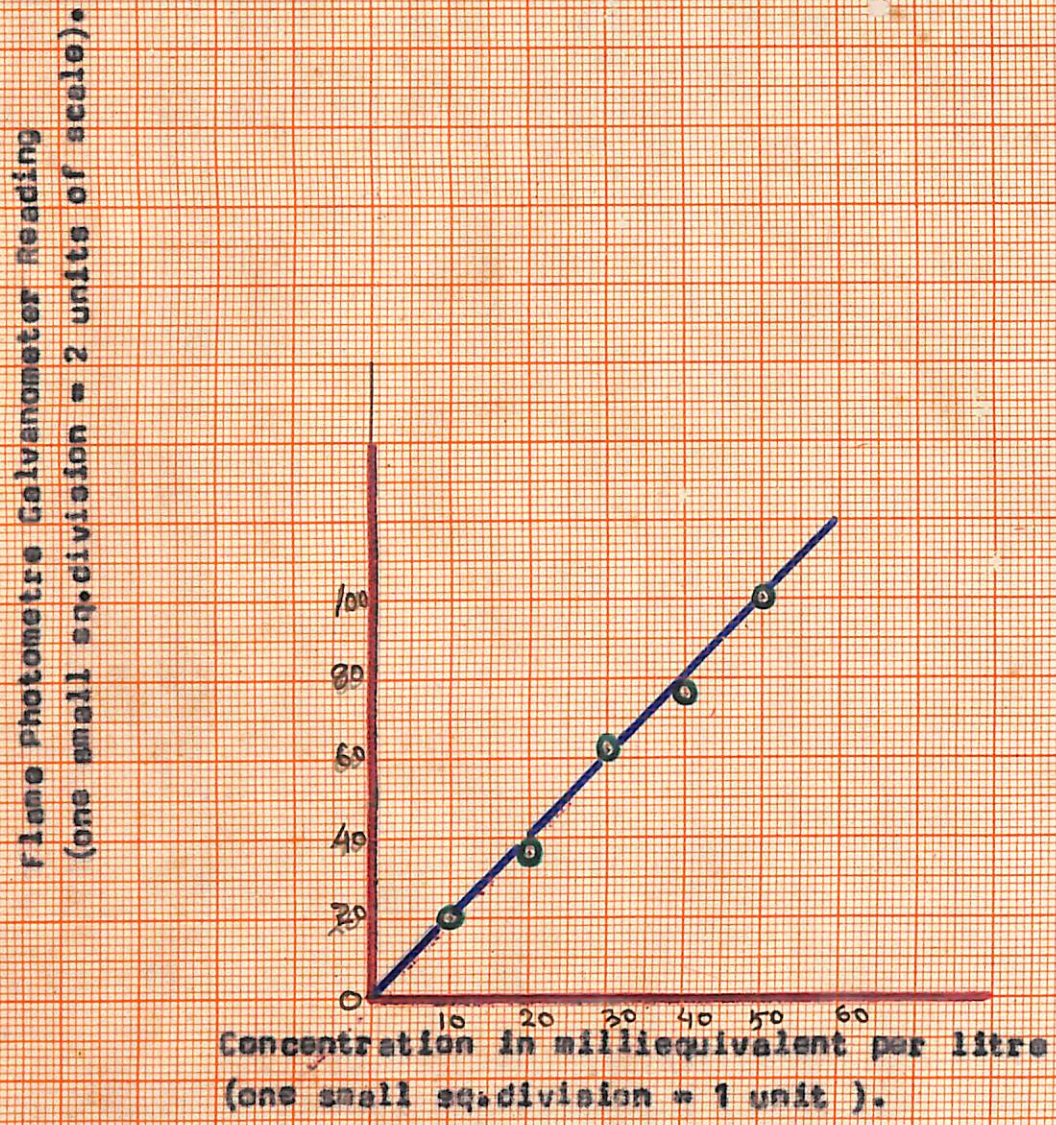


Fig.-3.

Histogram showing the level of Sodium and Potassium at 1st, 2nd and 3rd degree of uterine tone.

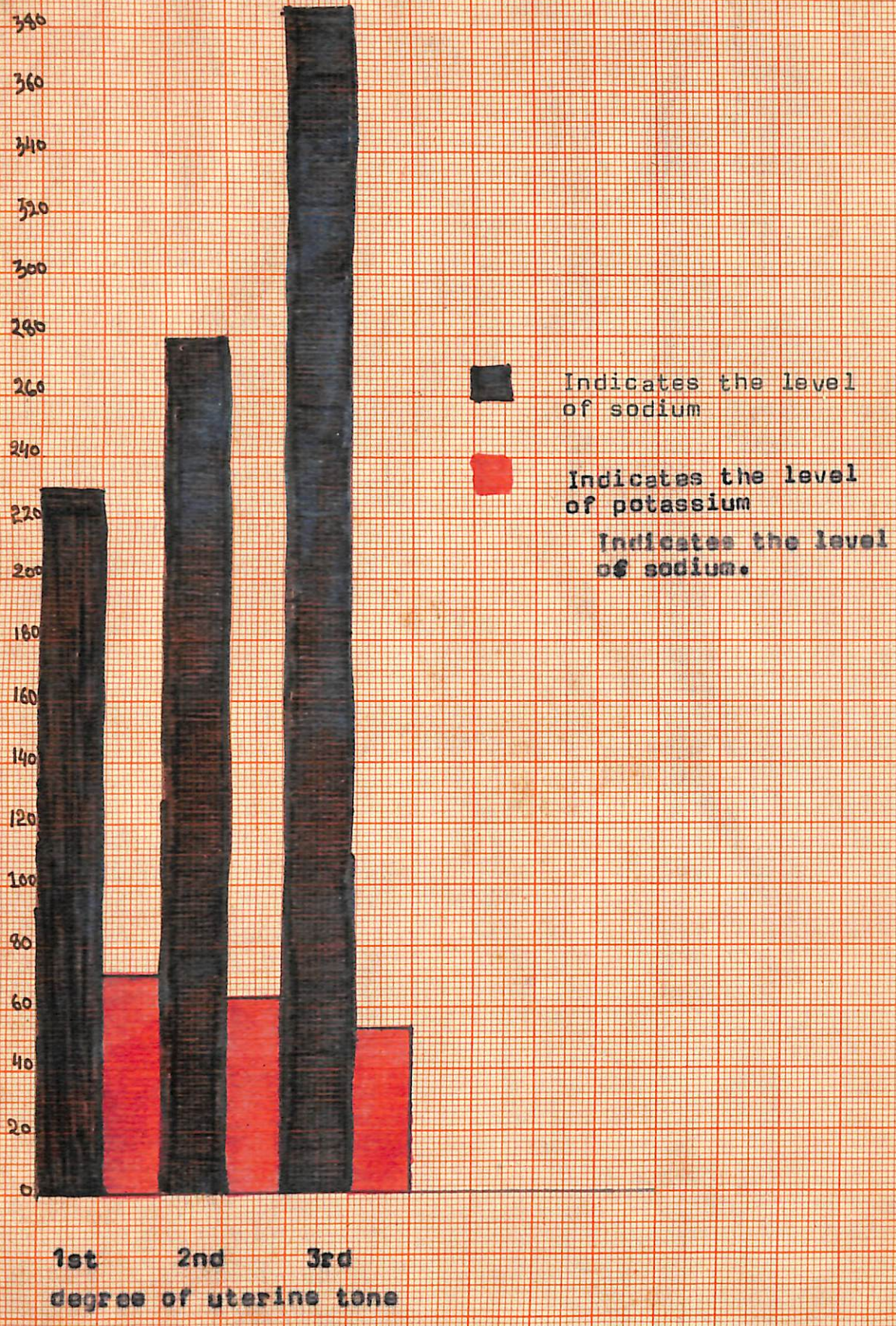
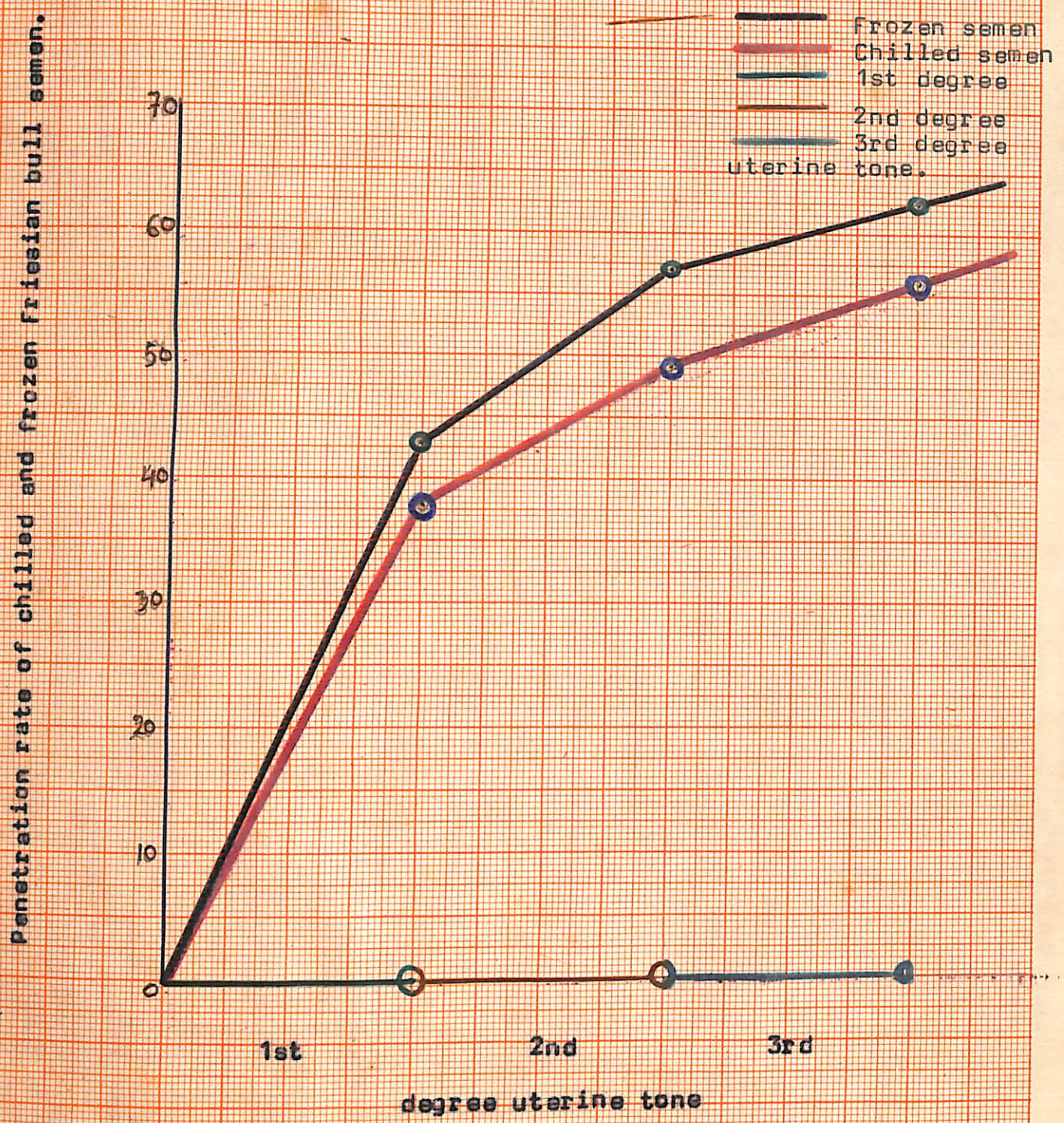


FIG-4

Graph showing the penetration rate of chilled and frozen friesian bull semen at 1st, 2nd and 3rd degree of uterine tone.



(Two small sq. division is equal to 1 (one) unit).

Fig. 5.

Histogram showing the penetration rate of chilled and frozen Friesian bull semen at 1st, 2nd and 3rd degree uterine tone.

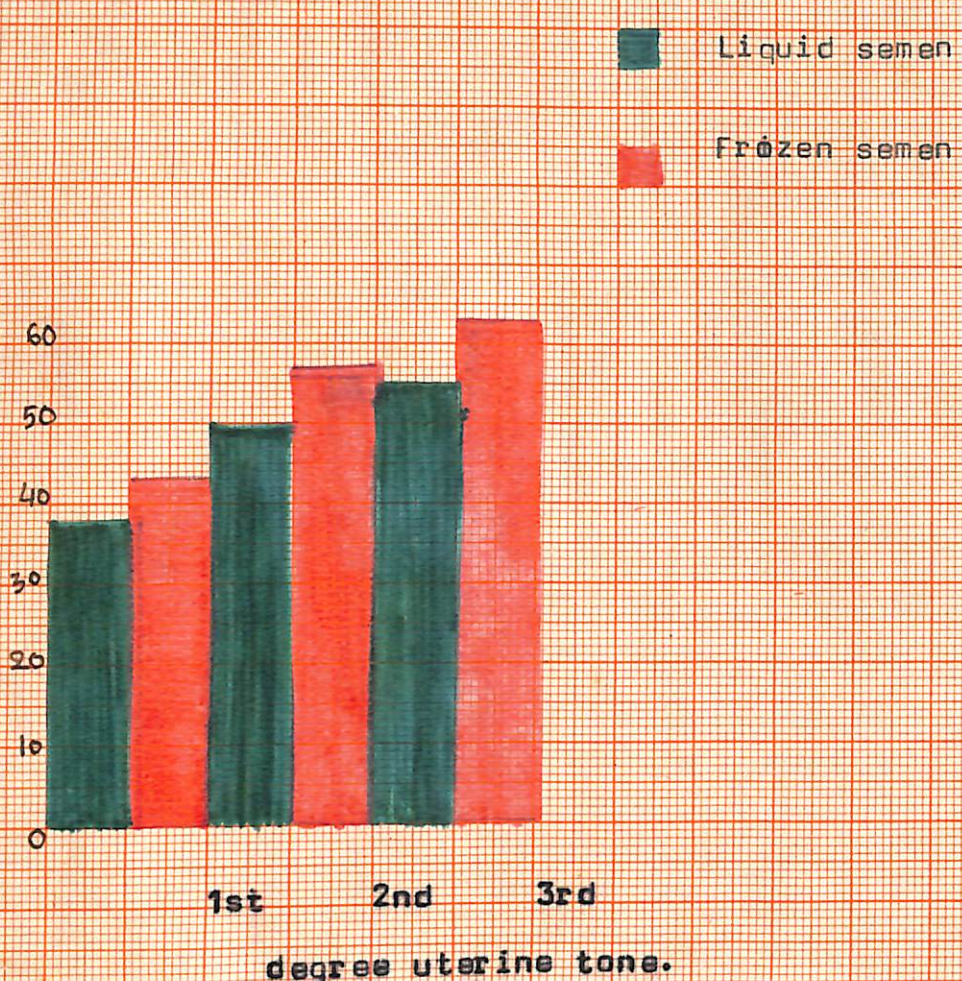


Table 7.

Showing the mean penetration rate of liquid Friesian bull semen in different groups of uterine tone during oestrum.

Intensity of uterine tone	n	Range	Penetration rate of liquid Friesian bull semen	
			Mean ± S.E.	C.V. %
1st degree	17	28-50	37.91±1.46	15.88
2nd degree	21	38.9-60.3	49.88±1.44	13.27
3rd degree	29	44.4-65.8	55.67±1.12	10.84

The penetration rate of liquid semen of Friesian bull in the oestrual mucus of cows increased with the increased intensity of the uterine tone. The average difference of liquid semen of Friesian bull penetration rate into the oestrual mucus between the different degrees of uterine tone was found to significant on statistical analysis at 1 per cent level (Table 8).

Table -8.

Showing the analysis of variance of the penetration rate of liquid semen of Friesian bull into the oestrual mucus at different degrees of uterine tone during oestrus.

Source of variance	d.f.	M.S.	F
Between degree of tone	2	1370.9	11.69 ++
Within degree of tone	64	117.25	

++ denotes significant at 1 per cent level.

The critical difference test as regards the difference in mean penetration rates of liquid Friesian bull semen recorded in first and third degree (15.96), first and second

degree (11.97) and second and third degree (3.99) uterine tone were found to be statistically significant at 1 per cent level (Table 9).

Table-9.

Showing the critical difference of liquid Friesian bull semen penetration rate between different uterine tones.

Comparison between intensities of uterine tone.	Mean liquid Friesian bull semen penetration between groups of uterine tone.	Critical difference at 5% level	Critical difference at 1% level
1st degree tone Vrs 3rd degree tone.	15.96 ++	1.94	2.58
1st degree tone Vrs 2nd degree tone.	11.97 ++	2.14	2.84
2nd degree tone Vrs 3rd degree tone	3.99 ++	1.86	2.47

++ significante at 1 per cent level.

Penetration rate of frozen semen of Friesian bull into the oestrual mucus of cows at different degrees of uterine tone.

The mean penetration rate of frozen semen of Friesian bull in the oestrual mucus collected during first, second and third degrees of uterine tone was found to be 42.63 $\pm$ 1.94, 57.21$\pm$1.29 and 62.92$\pm$7.32 milimeter/20 minutes respectively and are presented in Table - 10.

Table-10.

Showing the mean penetration rate of frozen semen of Friesian bull in the oestrual mucus collected at different degree of uterine tone during oestrum.

Intensity of uterine tone	n	Range	Penetration rate of frozen Friesian bull semen into the oestrual mucus	
			Mean ± S.E.	C.V.%
1st degree	17	30.8-58.9	42.63±1.94	18.78
2nd degree	21	37-64.2	57.21±1.29	10.38
3rd degree	29	59.2-68.1	62.82±7.32	62.70

The penetration rate of frozen semen of Friesian bull in the oestrual mucus of cows increased with the increased degree of uterine tone. The average difference of frozen Friesian bull semen penetration in the oestrual mucus between the different intensities of uterine tone was observed to be statistically significant at 1 per cent level (Table 11).

Table-11.

Showing the analysis of variance of the penetration rate of frozen semen of Friesian bull in the oestrual mucus at different degrees of uterine tone during oestrus.

Source of variance	d.f.	M.S.	F.
Between degrees of tone	2	2208.29	74.958 ++
Within degrees of tone	64	29.46	

++ significant at 1 per cent level.

The critical difference test as regards the difference in the penetration rates of frozen semen of Friesian bull between first and third degrees (20.19), first and second degrees (14.58) of uterine tone were found to be statistically significant at 1 per cent level whereas penetration rate values between second and third degree (5.61) uterine tone was significant at 5 per cent level (Table 12).

Table-12.

Showing the critical difference of frozen semen of Friesian bull penetration rate at different intensities of uterine tone.

Comparison between intensities of uterine tone	Mean frozen Friesian bull semen penetration rate at different degrees of uterine tone	Critical difference	
		at 5% level	at 1% level
1st degree tone Vrs 3rd degree tone	15.96 ++	1.94	2.58
1st degree tone Vrs 2nd degree tone	11.97 ++	2.14	2.84
2nd degree tone Vrs 3rd degree tone	3.99 ++	1.86	2.47

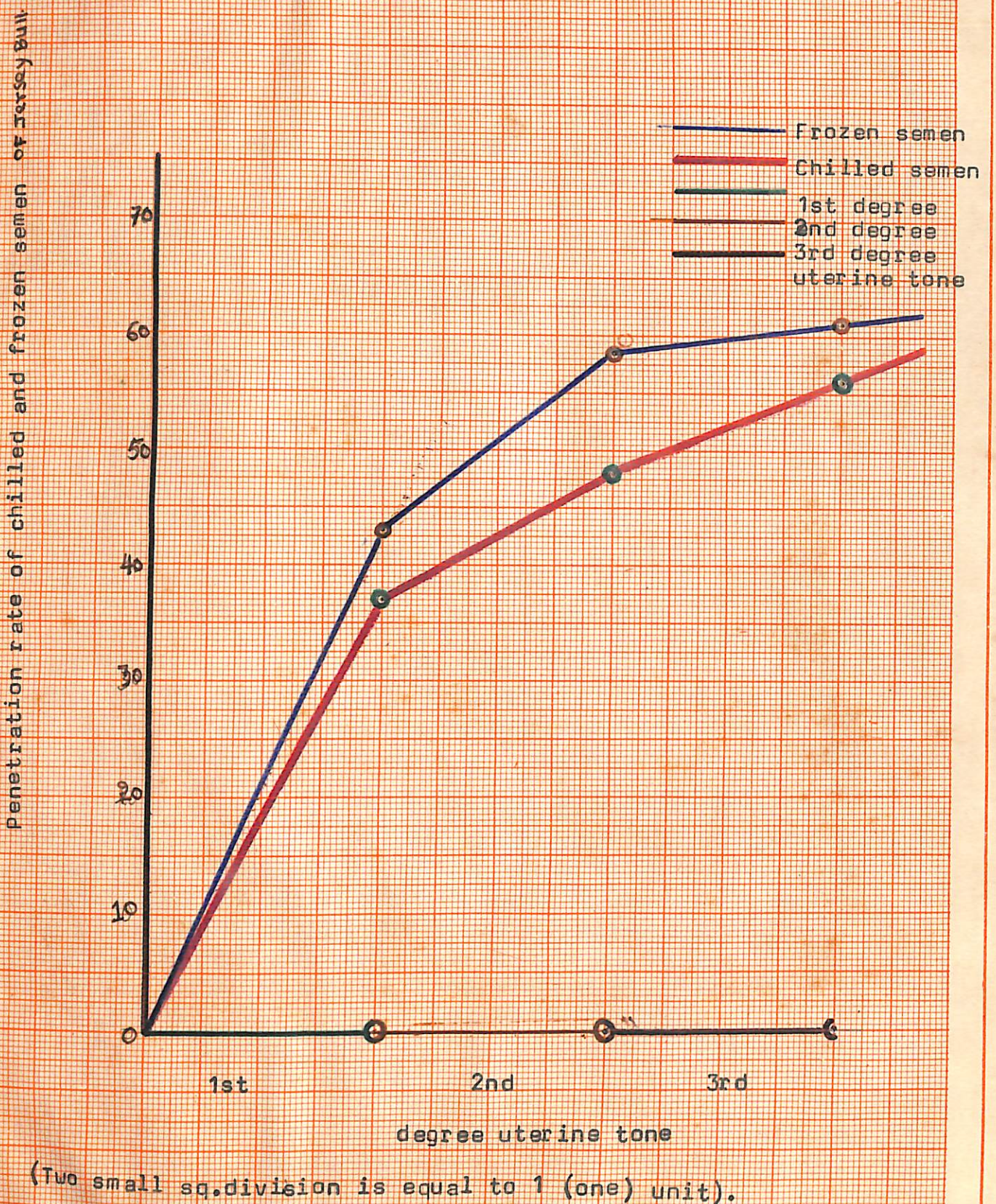
++ significant at 1 per cent level.

Penetration rate of liquid semen of Jersey bull in the oestrual mucus of cows at different degrees of uterine tone.

The mean value of penetration rate of liquid Jersey bull semen into the oestrual mucus of first, second and third degrres of uterine tone was found to be 37.5±1.67, 48.7±1.31 and 55.88±1.27 milimeter/20 minutes respectively (Table 13).

FIG- 6

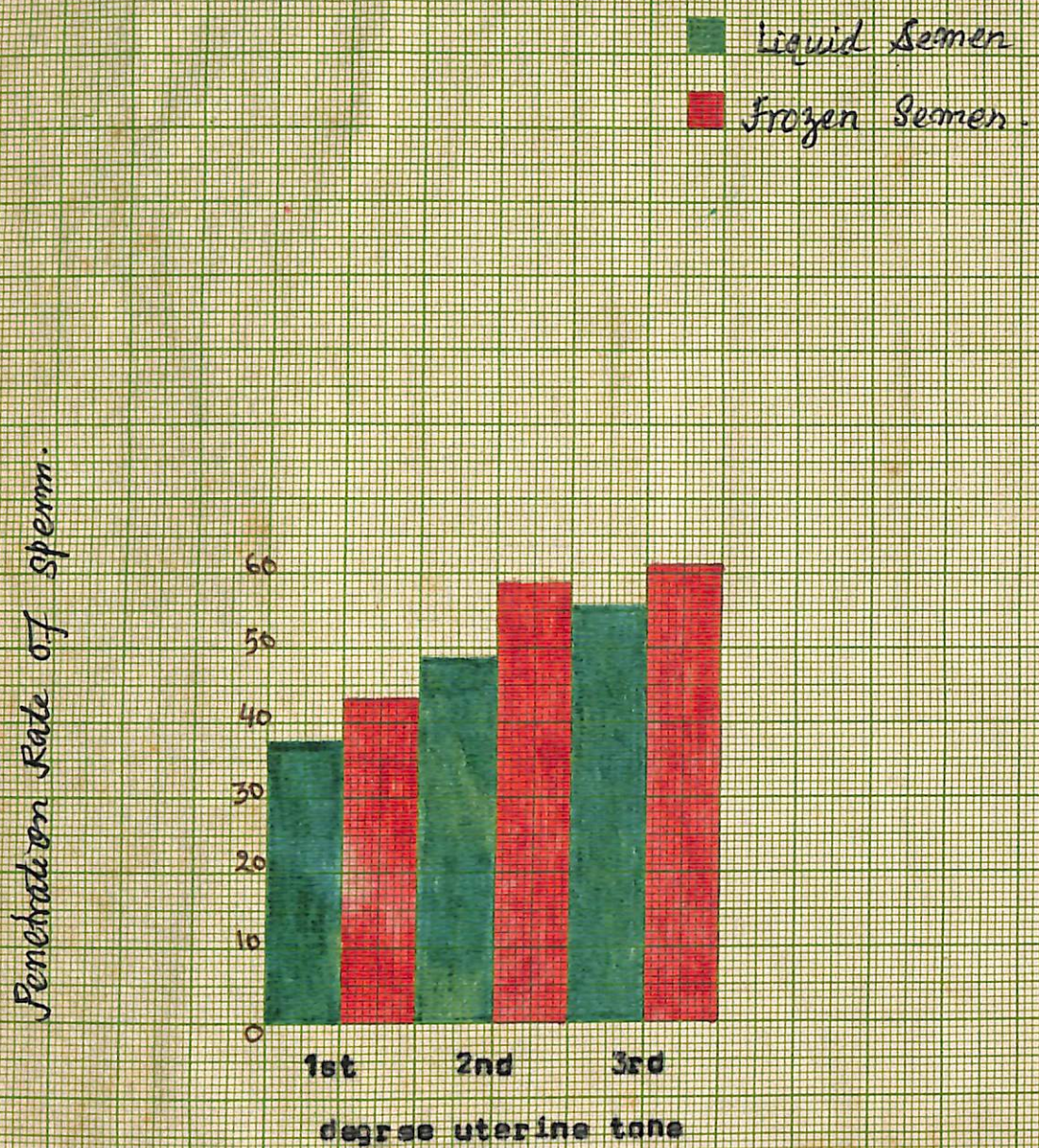
Graph showing the penetration rate of chilled and frozen bull semen at 1st, 2nd and 3rd degree of uterine tone.



(Two small sq.division is equal to 1 (one) unit).

Fig. 7.

Histogram showing the penetration rate of chilled and frozen Jersey bull semen at 1st, 2nd and 3rd degree of uterine tone



1 small Sq. eq = 1 mm.

Table-13.

Showing the mean penetration rate of liquid Jersey bull semen into oestrual mucus in different degrees of uterine tone during oestrum.

Degree of tone	n	Range	Penetration rate of liquid Jersey bull semen into oestrual mucus of cows	
			Mean ± S.E.	C.V.%
1st degree	17	30-54.1	37.5 ± 1.67	18.42
2nd degree	21	39.6-58.32	48.7 ± 1.31	12.40
3rd degree	29	44.0-65.62	55.88 ± 1.27	12.27

The penetration rate of liquid semen of Jersey bull in the oestrual mucus of cows increased in accordance with the increased degree of the uterine tone. The average difference of liquid Jersey bull semen penetration rate into the oestrual mucus between the groups of uterine tone was significant at 1 per cent level (Table-14).

Table-14.

Showing the analysis of variance of the penetration rate of liquid Jersey bull semen in the oestrual mucus of cows at different degrees of uterine tone during oestrum.

Source of variance	d.f.	M.S.	F
Between degrees of tone	2	1631.931	37.13 ++
Within degrees of tone	64	43.95	

++ significant at 1 per cent level.

The critical difference test as regards the difference in liquid Jersey bull semen penetration values between first and third degrees (18.38), first and second degrees (11.20)

and second and third degrees (7.1) of uterine tone were found to be significant at 1 per cent level (Table-15).

Table-15.

Showing the critical difference of liquid Jersey bull semen penetration rate between different degrees of uterine tone.

Comparison between degrees of uterine tone	Mean liquid Jersey bull semen penetration rate difference between groups of tone	Critical difference at 5% level	Critical difference at 1% level
1st degree tone Vrs 3rd degree tone	18.38 ++	3.90	5.187
1st degree tone Vrs 2nd degree tone	11.20 ++	4.30	5.719
2nd degree tone Vrs 3rd degree tone	7.1 ++	3.76	5.008

++ Significant at 1 per cent level.

Penetration rate of frozen semen of Jersey bull in the oestrual mucus of cows at different degrees of uterine tone.

The mean penetration rate of frozen semen of Jersey bull in the oestrual mucus at first, second and third degrees of uterine tone was found to be 43.05 ± 1.70, 58.86 ± 0.74 and 61.33 ± 3.15 milimeter/ 20 minutes respectively (Table-16).

Table-16.

Showing the mean penetration rate of frozen semen of Jersey bull into the oestrual mucus in different groups of uterine tone during oestrus.

Degree of tone	n	Range	Penetration rate of frozen Jersey bull semen into the oestrual mucus of cows.	
			Mean ± S.E.	C.V.%
1st degree	17	32.5-56.3	43.05 ± 1.70	16.27
2nd degree	21	42.0-63.1	58.86 ± 0.74	5.82
3rd degree	29	59.6-68.5	61.33 ± 3.15	27.66

The penetration rate of frozen semen of Jersey bull increased with the increased intensity of uterine tone. The average difference of frozen semen of Jersey bull into the oestrual mucus between the degrees of uterine tone was statistically significant at 1 per cent level (Table-17).

Table-17.

Showing the analysis of variance of the penetration rate of frozen Friesian bull semen into the oestrual mucus of cows at different degrees of uterine tone during heat.

Source of variance	d.f.	M.S.	F.
Between degrees of tone	2	1923.775	13.552 ++
Within degrees of tone	64	141.953	

++ significant at 1 per cent of level.

The critical difference test regarding the difference in frozen semen of Jersey bull penetration values between first and third degrees (18.28), first and second degrees (15.81) of uterine tone were found to be significant at 1 per cent level. But the penetration rate between second and third degree (2.47) uterine tone was found to be

statistically non-significant (Table-18).

Table-18.

Showing the critical difference of frozen semen of Jersey bull penetration rate between different degrees of uterine tone.

Comparison between degrees of uterine tone	Mean frozen Jersey bull semen penetration rate difference between groups of tone	Critical difference at 5% level	Critical difference at 1% level
1st degree tone Vrs 3rd degree tone	18.28 ++	7.02	9.33
1st degree tone Vrs 2nd degree tone	15.81 ++	7.72	0.26
2nd degree tone Vrs 3rd degree tone	2.47 N.S.	6.72	8.94

++ Significant at 1 per cent level.

N.S. non-significant.

pH of oestrual mucus (during oestrus)

In the present study the mean value of pH of oestrual mucus of cows was found to be 7.92 ± 0.064 (Table-19).

Table -19.

Showing the pH of oestrual mucus of cows.

No. of cows in heat	Range	pH of oestrual mucus Mean ± S.E.	C.V.%
67	7.3-8.6	7.92 ± 0.064	0.808

Penetration rate of chilled and frozen semen of Friesian and Jersey bull in the oestrual mucus.

In the present study the average penetration rate of chilled and frozen semen of Friesian bull was found to be 50.59 ± 1.13 and 57.30 ± 1.26 mm/20 minutes respectively whereas the penetration rate in case of Jersey chilled and frozen semen was 49.90 ± 1.023 and 58.58 ± 2.085 mm/20 minutes respectively (Table-20).

Table-20.

Showing the average penetration rate of chilled and frozen Friesian and Jersey bull semen mm/20 minutes into the oestrual mucus of cows.

Breeds	n	Penetration rate into the oestrual mucus of cows mm/20 minutes			
		Liquid		Frozen	
		Mean ± S.E.	C.V.%	Mean ± S.E.	C.V.%
Friesian	67	50.59±1.13	18.23	57.30±1.26	18.04
Jersey	67	49.90±1.023	16.78	58.58±2.085	29.13

Analysis of variance was carried out to find out the effect of breeds as well as types of semen on the migration rate. It was found that breeds did not seem to have any significant effect on the migration rate of sperm. The difference between types of semen (liquid and frozen) were found to be statistically significant at 1 per cent level (Table-21).

Table-21

Showing the analysis of variance of breeds and types of semen on the penetration rate of sperm into the oestrual mucus of cows.

Source of variance	d.f.	M.S.
Between breeds	1	5.82 N.S.
Between types of semen	1	3965.52 ++
Error	265	24.86

++ denotes significant at 1 per cent level.

N.S. denotes non-significance.

Arborisation pattern of oestrual mucus.

Out of 67 samples of oestrual mucus studied, typical fern tree crystallisation pattern was found in 65 samples. In typical type of arborisation the fern tree like pattern was quite clear. In atypical type of arborisation the pattern appeared disorganised (Table-22).

Table-22.

Showing the percentage of typical and atypical types of arborisation pattern in the oestrual mucus of cows.

Types of arborisation pattern	n	Percentage of typical and atypical types of crystallisation pattern
Typical	65	103.076
Atypical	2	1.34

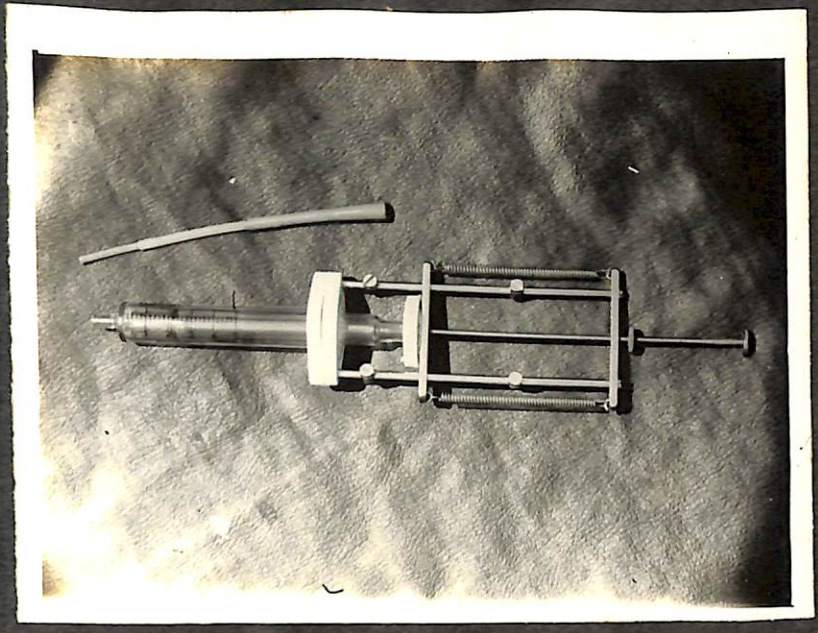


Fig. 4 : Showing SPROJTE DL syringe pipette of 5 ml capacity (DANSK LABORATORIEUDSTYRA/S RYESGADE 3. 2200 KOSENHAVN, N.TIE (01) 392600).

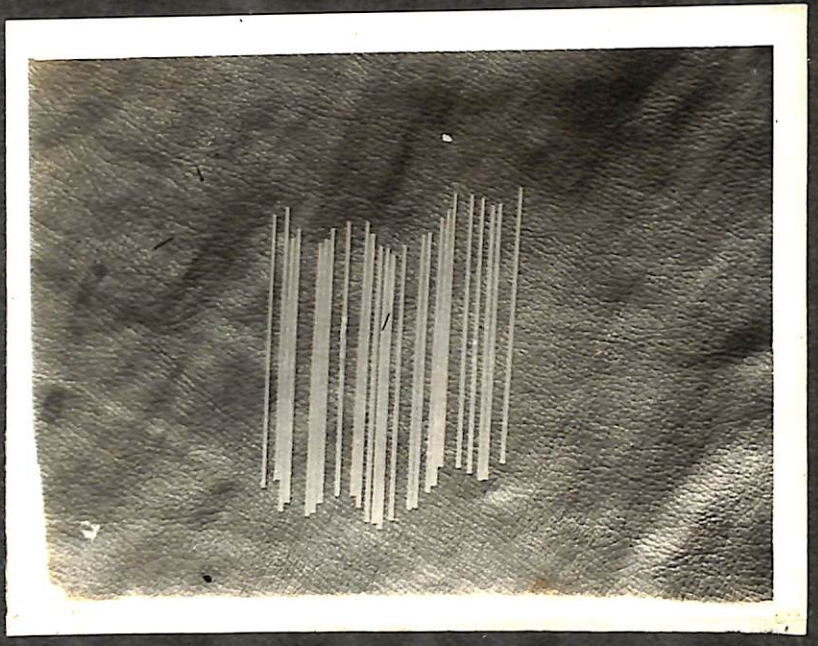


Fig. 5 : Showing select capillary tube(plain). Propper Manufacturing Co.,INC. Long. Island City, New York.

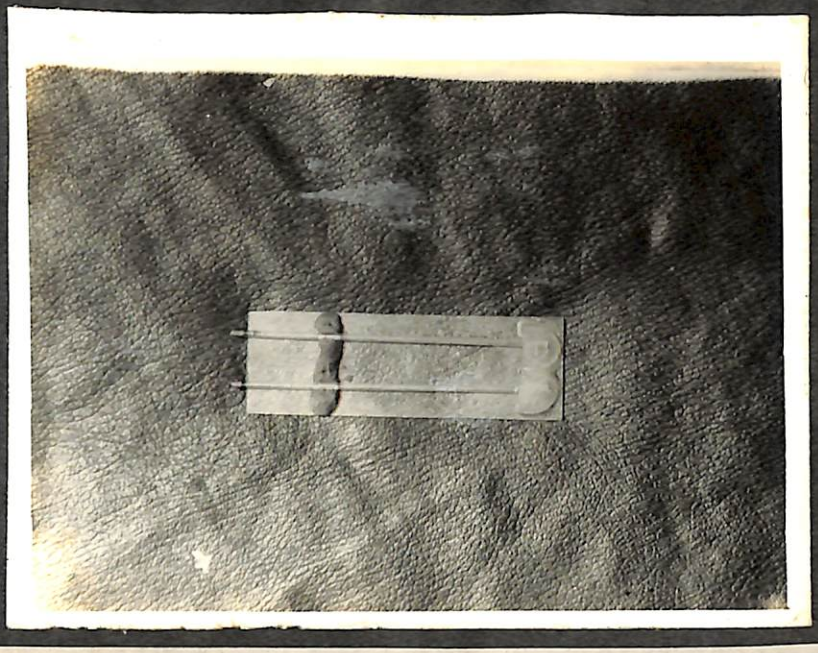
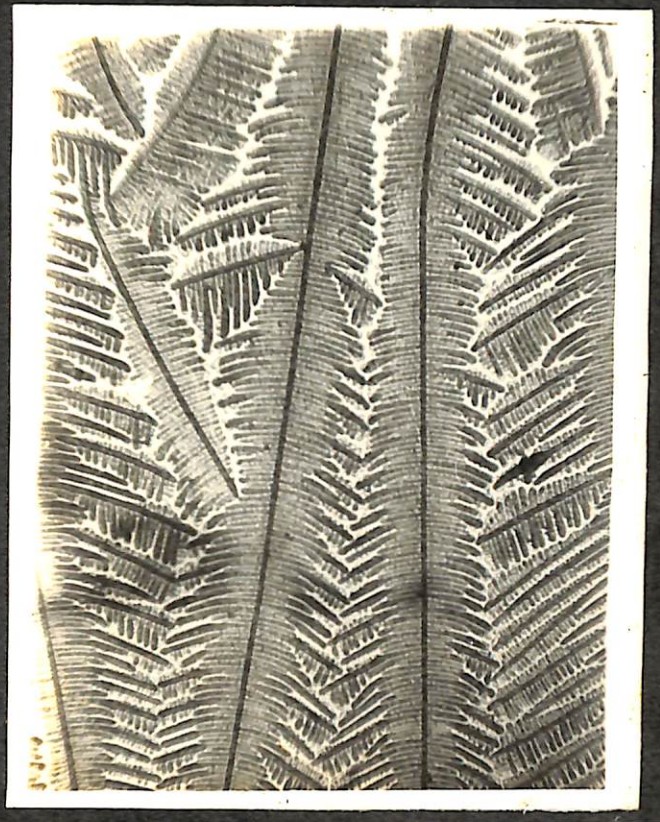


Fig. 6 : Showing specially ment slide with capillary tube adjusted for sperm penetration.



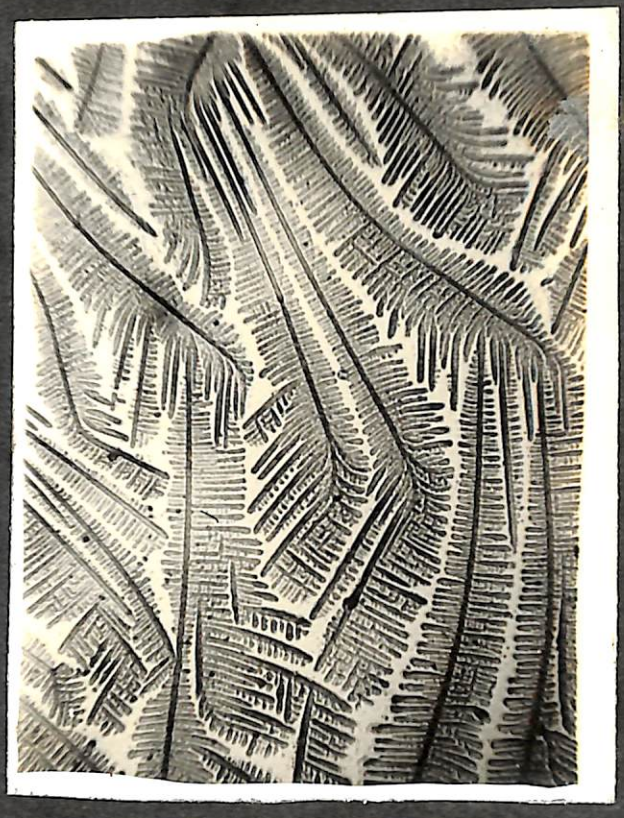


Fig. 7 : Typical type of fern like crystallisation pattern of oestrual mucus.

Fig. 8 : Typical type of fern like crystallisation pattern of oestrual mucus.

D I S C U S S I O N

DISCUSSION

Uterine tone.

The present study embraced 67 cows which were brought to the out-door clinics of Gynaecology department of Bihar Veterinary College, Patna for insemination. In each cow, the changes in the genital organs were monitored by rectal palpation to categorise them into first, second and third degrees of uterine tone. Out of 67 cows, 29 were categorised to have pronounced signs of heat with third degree uterine tone.

The uterus becomes firm, errect and turgid during oestrus (Hukeri, 1965). The tonicity of uterus depends upon the interaction of uterus and estradiol. Concurrently, estradiol stimulates the secretion of mucus and regulates the level of electrolytes in the genital tract secretion. There is a positive correlation with the uterine tone to the level of sodium. When the tonicity of uterus is maximum, the concentration of sodium is highest in oestrual mucus, whereas, the tonicity of uterus decreases the concentration of potassium. Therefore, a negative correlation was found between sodium and potassium level of oestrual mucus (Table 1, 4 and Fig.-I) and the aforesaid facts have been substantiated in the present study.

During the follicular phase of the estrous cycle the vascularity of the reproductive tract increases owing to the action of estradiol. The prolonged effect of the hormone

is responsible for the development of the numerous arterioles of the endometrium. The increase in vascularity begins during proestrus, during estrus it reaches to maximum and the number of arterioles drop rapidly after ovulation.

The difference in the tonicity of the uterus as recorded in this study depended to a great extent at which stage of estrus the cases were brought to the clinics. The third degree tone of the uterus was recorded in those cases which were brought during middle to end of estrus. This period being the best time for insemination coincided with the maximum tonicity of uterus. The difference in the tonicity of uterus in small percentage of cases might be attributable to level of estradiol, the responsiveness of the target organs and endocrine constitution of the animal.

The results of present study are thus consistent with the findings of Hukeri (1965) and Sinha (1969).

Level of sodium in oestrual mucus of cows.

The average levels of sodium in oestrual mucus of cows collected at first, second and third degrees of uterine tone were 228.90, 278.40 and 386.40 mg/100 ml (Table-1). The findings of present study are in agreement with the results of Gupta (1962), Guay (1966a, 1966b), Gupta et al. (1962), Reddy (1974), Shoaib (1975) and Wani et al. (1979).

The tonicity of the uterus determined the level of sodium and its level varied according to different tones which on statistical analysis was found to be significant (Fig.-I).

The results of present study are at variance with the findings of Guay and Lamothe (1970) as they have reported the mean sodium value of bovine cervical mucus to be 543.12 mg/100 ml. The level is higher than the findings of present study. The variation in the level of sodium of oestrual mucus might be due to the various factors like the collection of mucus at different timings of oestrus, breed, age, technique of sodium estimation and hormonal status of the animals.

Level of potassium in the bovine oestrual mucus.

The average level of potassium in the oestrual mucus of cows collected at first, second and third degrees of uterine tone was found to be 70.24, 63.90 and 53.87 mg/ 100 ml respectively (Table-4). The results are in close accordance with the observations of Gupta (1962), Gupta et al. (1962), Guay (1966a-1966b), Guay and Lamothe (1970), Reddy (1974), Shoaib (1975) and Wani et al. (1970). The little variation between the result of present study and observations reported by different workers might be due to collection of oestrual mucus at different degrees of uterine tone, age, breed, nutritional practices, technique of potassium estimation and the level of hormones of the animals.

Interestingly, there existed a negative correlation between the levels of sodium and potassium estimated in the samples of oestrual mucus collected (Fig.-I) at different degrees of uterine tone. The level of these ions thus reflected the tonicity of the uterus and indirectly also the stage of heat period.

Penetration rate of chilled and frozen semen of Jersey and Friesian bulls in the oestrual mucus of cows.

The average penetration rate of liquid semen of Jersey bull in the oestrual mucus of cows was found to be 37.5 millimeter/20 minutes (Table-13). The penetration rate of frozen semen of Jersey bull was found to be 40.05 mm/20 minutes in the oestrual mucus collected at first degree of uterine tone (Table-16). The penetration rate of liquid semen of Jersey bull in the oestrual mucus collected during second and third degree of uterine tone was found to be maximum 48.7 $\pm$ 1.31 mm and 55.88 $\pm$ 1.27 mm/20 minutes respectively (Table-13 and Fig.-II). The mean migration rate of frozen semen of Jersey bull in the oestrual mucus collected at second and third degrees of uterine tones was 58.86 mm/120 minutes and 61 mm/120 minutes respectively.

The average penetration rate of chilled and frozen semen of Friesian bull in the oestrual mucus of cows collected during first, second and third degrees of uterine tone was found to be 37.91 mm (at first degree), 49.88 mm (at second) and 55.67 mm (at third degree) by

liquid (Friesian bull) semen and 42.65 mm (at first), 57.21 mm (at second) and 62.82 mm (at third degree) by frozen (Friesian bull) semen /20 minutes respectively (Table-7).

The average penetration rate of chilled and frozen semen of Jersey and Friesian bulls in the bovine oestrual mucus was observed to be 49.90 mm, 58.58 mm (migration rate of Jersey bull semen) and 50.59 mm 57.30 mm/20 minutes respectively (migration rate of Friesian bull semen).

The results of the present study are in accordance with the findings of Roark and Harman (1950), Moellar and VanDemark (1955), Tampion and Gibbons (1962), Akhtar (1973) and Hallet and Craig (1979). The little variation observed by the different workers regarding the penetration rate might be due to the collection of oestrual mucus samples at different timings of oestrous, difference in the technique of study and breed, age and hormonal constitution of the animals.

The highest penetration rate of spermatozoa was observed in the oestrual mucus collected during the third degree of uterine tone (middle to end of oestrus period). The viscosity of the mucus was thin, ropy and easily aspirable. The observations are comparable with the finding of Roark and Herman, (1950).

Moellar and VanDemark (1955) reported significant differences in the speed of spermatozoa in different ejaculates of the same bull. Roslanowski and Koefee-Jonanson (1959) observed a positive correlation between

follicular development, viability and speed of spermatozoa in cervical mucus collected at 12 hour intervals during oestrum. Results of the present study also substantiate the findings of previous workers (loc cit). It may be due to the low viscosity of mucus collected during high intensity of the uterine tone. Harvey (1960) correlated the average speed of human spermatoza with fertility index. The human sperm with higher penetration rate was supposed to have the best fertility index.

The penetration rate of liquid semen of Friesian bull was found to be 37.91 mm, 49.88 mm and 55.67 mm/20 minutes at the sodium level of bovine oestrual mucus to be 228.90 mg, 278.40 mg and 386.40 mg/100 ml respectively (Table-7 and 1). The penetration rate of frozen semen of Friesian bull was observed to be 42.63 mm, 57.21 mm and 62.82 mm/20 minutes (Table-10) at the same sodium concentration of oestrual mucus as in case of liquid semen. It is evident from the table-20 that the penetration rate of frozen semen samples of both Friesian and Jersey bulls was comparatively more than the chilled semen samples. In this respect, it is concievable that as the metabolic activity of the frozen semen samples are quite restricted they have all the energy reserve intact and as such they were more vigorous in penetrating the mucus samples. The chilled semen samples which were studied approximately after 24 hours of preservation, the sperm might have lost their energy and vigour as the metabolic activity is not kept restricted.

The level of potassium in the oestrual mucus at first, second and third degree of uterine tone was recorded to be 70.24 mg, 63.90 mg and 53.87 mg/100 ml respectively (Table-4).

In this study it was found that the penetration rate of spermatozoa was maximum (60.82 mm/20 minute) when the level of sodium was 386.40 mg/100 ml of oestrual mucus and that of potassium was 53.87 mg/100 ml (Table 1 and 4). From the table it is evident that high level of sodium and low level of potassium facilitated the penetration rate of spermatozoa.

The results of the present study are in accordance with the findings of Guay (1966a-1966b). Who reported that the high level of sodium and low level of potassium in the oestrual mucus increased the penetration rate of spermatozoa whereas the low level of sodium and high level of potassium of oestrual mucus decreased the penetration rate.

Reddy (1974) reported the mean penetration rate of spermatozoa in the bovine oestrual mucus to be 43.03 $\pm$ 2.8 mm/20 minute at the sodium and potassium level of oestrual mucus 361.82 $\pm$ 11.8mg and 55.83 $\pm$ 4.32 mg/100 ml. The observations of Reddy (1974) are consistent with the results of present study.

It is, therefore, conceivable that spermatozoa penetrate easily when the level of sodium is high in oestrual mucus. The level of sodium was recorded to be high in the oestrual mucus samples collected during third degree uterine tone. The results of the present study do indicate that there exists great correlation between the penetration rate of spermatozoa and levels of sodium and potassium in the oestrual mucus. The penetration rate of sodium is high whereas the penetration is reduced when the level of potassium tends to go high.

The oestrual mucus seems to be significant in many aspects of reproductive physiology, the primary functions are to protect the uterine cavity and to control sperm penetration through the cervix. The production of mucus with certain changes in its physical properties depends upon the hormonal levels.

During the preovulatory period of oestrous cycle, the level of estrogen stimulates the production of mucus, the secretion is profuse, thin, clear and favourable for sperm penetration. But after ovulation or in the luteal phase the secretion becomes thick, scanty, opaque and unfavourable for sperm penetration. The present evidence also suggests that the structure elements of the mucus gel consists of long threads like glycoprotein molecule with a high degree of branching dependent on the active molecules and biochemical alteration in its content during the oestrus cycle may have effects upon spermatozoal penetration through

changes in the viscosity of the oestrual mucus (Iacobelli, 1971).

Oestrual mucus consists of macromolecules of mucin of epithelial origin, which are composed of glycoproteins (particularly sialomucin type) which contain some 25 per cent amino acids and 75 per cent carbohydrates. The mucin is made of a long, continuous polypeptide chain with numerous oligosaccharide side chains.

After mating, spermatozoa are oriented toward the internal os. As the flagellum beats and vibrates the sperm head is propelled forward in the channels of least resistance. The micro and microheologic properties of cervical mucus play a major role in sperm migration. Sperm penetrability increases with the cleanliness of mucus, since cellular debris and leucocytes delay sperm migration. The aqueous spaces between the micelles allow the passage of sperm as well as diffusion of soluble substances. Proteolytic enzymes may hydrolyze the back-bone protein or some of the cross-linkages of the mucin and reduce the network to a less resistant mesh with more open channels for the passage of sperm migration. When oestrual mucus and semen are placed in apposition in vitro, phase lines immediately occur between the two fluids. Sperm phalanges soon apear and develop marked degrees of arborization, the terminal aspects of which consist of channels through which one or two spermatozoa can pass (Hafez, 1975).

pH value of the oestrual mucus.

The average pH recorded during present study was 7.92 ± 0.064. The result was in close agreement with the observations of most of the previous workers including Chaudhuri and Prasad (1954), Gupta (1962), Hukeri (1965), Akhtar (1973) and Luktuke (1974).

The findings are at variance with that of Hartwig (1959) who reported the value of pH to be 6.12 while Bacic (1962) found the pH to be 6.9. Zust (1966), Kouser (1969) and Wani et al. (1979) reported the average pH to be 6.4, 6.21 and 6.97 of bovine oestrual mucus respectively.

The variation in pH values of oestrual mucus as reported by different workers (loc cit) might be attributable to difference in the breed, age, nutritional status and difference in the stages of oestrus during which mucus samples were collected. The technique followed in estimation of pH may also be responsible for variation in the pH values.

Arborisation pattern of oestrual mucus.

Crystallisation pattern of oestrual mucus has great importance in detection of oestrus and the phases of oestrus cycle. Due to the unique biophysical characteristics, the oestrual mucus has several rheologic properties such as ferning (crystallisation in fern leaf-like shape upon drying), elasticity, viscocity, thixotropy and tack (stickiness). The secretion of oestrual mucus is stimulated

by ovarian estrogen and inhibited by progestone.

According to Scott Blair and Glover (1957), the formation of crystallisation pattern is due to the presence of sodium chloride and mucin content of the oestrual mucus Later on, De Vuyst et al. (1961) reported three more factors responsible for the composition of fern-like crystallisation and the contents were mucoprotein, sodium and potassium chloride. The water content of arborisation pattern was found to increase under the influence of folliculin.

Out of 67 oestrual mucus samples collected to study arborisation pattern in the present study, 65 were found to be typical and two were found to be atypical. The fern-like crystallisation patterns obtained in the present study are comparable with the findings of various workers. (Higaki and Awai, 1953, Bone, 1954, Coluzzi and Battistacci, 1954, Pozolora, 1955, McDonald and Raesida, 1956, Scott Blair and Glover, 1957, Horvath, 1960, Howes et al., 1960, Luktuke and Subramaniam, 1961, Bane and Rajakoski, 1961, Sysoev, 1961, Gamcik and Sevčík, 1962, Senze and Zebracki, 1962, Abusineina, 1962, Mukeri, 1965, Prokopanov et al., 1965 and Zust, 1966). The typical type of arborisation pattern as recorded resembled with the work of Scott Blair and Glover (1954), Abusineina (1962), Kouser (1965) and Sinha (1969).

Bone (1954) reported that the arborisation pattern was related to the progesterone activity, whereas, Bane (1954), Pozolora (1955), Abusineia (1962) and Gamcik and Sevcik (1962) reported that the occurrence of crystallisation pattern was due to oestrogenic activity and was most pronounced during the follicular phase. During the luteal phase fern-like crystallisation may not be typical or may be absent due to the high level of progesterone. The most typical type of arborisation was found in those samples in which the viscosity of the mucus was thin and this has also been substantiated by Abusineina (1962), Hukeri (1965) and Sinha (1969).

SUMMARY

SUMMARY

The present study was conducted on 67 cows in heat brought for insemination at the out-door clinics of Gynaecology department of Bihar Veterinary College, Patna.

Before insemination, gynaecological examinations was done to ascertain the fitness of the cases for insemination. Due emphasis was laid to monitor the ovarian and uterine changes during oestrum by rectal palpation.

On the basis of uterine changes the tone of the uterus was classified as first, second and third degree. Besides the presence or absence of follicles on the ovaries, patency of portio and the nature of oestrual mucus discharge were also included in the examination.

Sixty seven oestrual mucus samples were collected for estimation of sodium, potassium, pH and penetration rate of frozen and liquid semen of Friesian and Jersey bulls. Study of arborisation pattern of oestrual mucus was also done.

In the oestrual mucus samples collected from the cases in which the uterine tone was of first degree, the average levels of sodium and potassium were found to be 228.90 ± 5.55 and 70.24 ± 1.77 mg/100 ml mucus. The mean penetration rates of frozen and liquid semen of Friesian and Jersey bulls in the oestrual mucus was observed to be 42.63 ± 1.94 mm, 37.90 ± 1.46 mm and 43.05 ± 1.70 mm, 37.5 ± 1.67 mm/20 minutes respectively.

In the oestrual mucus samples collected from cases in which the uterine tone was of second degree, the average level of sodium and potassium was found to be 278.40 ± 6.90 mg and 63.90 ± 2.07 mg/100 of mucus. The mean penetration rates of frozen and liquid semen of Friesian and Jersey bulls were observed to be 57.21 ± 1.29 mm, 49.88 ± 1.44 mm and 58.86 ± 0.74, 48.7 ± 1.31 mm/20 minutes respectively.

In the oestrual mucus samples collected from cases in which the uterine tone was of third degree, the average level of sodium and potassium was found to be 386.40 ± 5.25 mg and 53.87 ± 2.72 mg/100 ml of mucus samples. The mean penetration rates of frozen and liquid semen of Friesian and Jersey bulls in the oestrual mucus were recorded to be 62.82 ± 7.32 mm, 55.67 ± 1.12 mm and 61.33 ± 3.15 mm, 55.88 ± 1.27 mm/20 minutes respectively.

Statistically significant difference was found between the penetration rate of chilled and frozen semen. The penetration rate of frozen semen was observed to be higher than the chilled semen. The penetration rate of sperm was found to be dependent on the intensity of the uterine tone. The penetration rate of sperm was higher in the oestrual mucus samples collected from cases having highest intensity of uterine tone whereas it was lower in oestrual mucus samples collected from cases having low intensity of uterine tone.

The level of sodium in oestrual mucus was found to be highest in the mucus samples collected during the third

ee of uterine tone. The level of sodium was found to be
low st in the mucus samples collected at first degree
eri ne tone. Contrary to the level of sodium, the
of potassium in oestrual mucus was found to be
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sia n bulls. Correlations were found to be statist-
ign ificant.

pH of the oestrual mucus samples were found to
ne in nature. The pH range varied between 7.3 to
ver ge being 7.92 ± 0.064.

of 67 oestrual mucus samples studied for arbor-
tte rn 65 had typical and 2 had atypical type of
ti on pattern.

degree of uterine tone. The level of sodium was found to be the lowest in the mucus samples collected at first degree of uterine tone. Contrary to the level of sodium, the level of potassium in oestrual mucus was found to be highest in first degree and lowest in the third degree of uterine tone.

The levels of sodium and potassium of oestrual mucus had correlation with the degrees of the uterine tone and the penetration rate of chilled and frozen semen of Jersey and Friesian bulls. Correlations were found to be statistically significant.

The pH of the oestrual mucus samples were found to be alkaline in nature. The pH range varied between 7.3 to 8.6 the average being 7.92 ± 0.064.

Out of 67 oestrual mucus samples studied for arborisation pattern, 65 had typical and 2 had atypical type of crystallisation pattern.

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