VOL. 5 No. 1 : JUNE 1984

The Indian Journal of Animal Reproduction

JOURNAL OF THE
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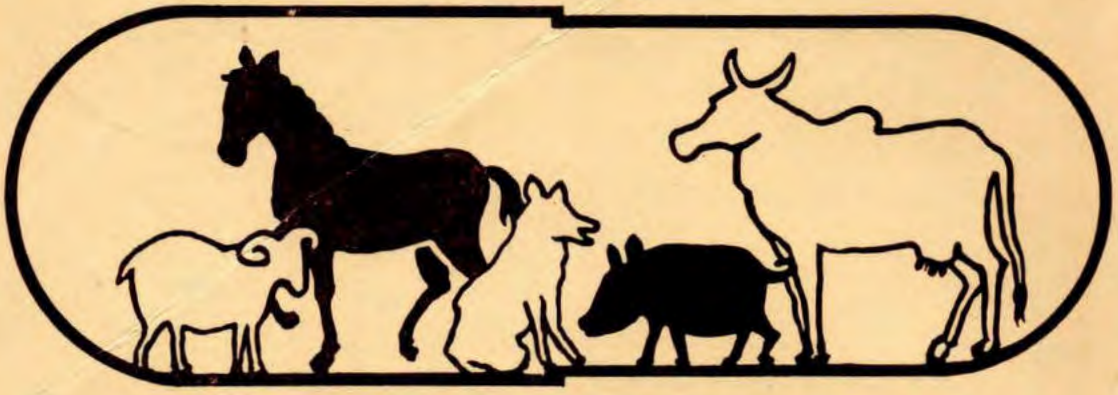

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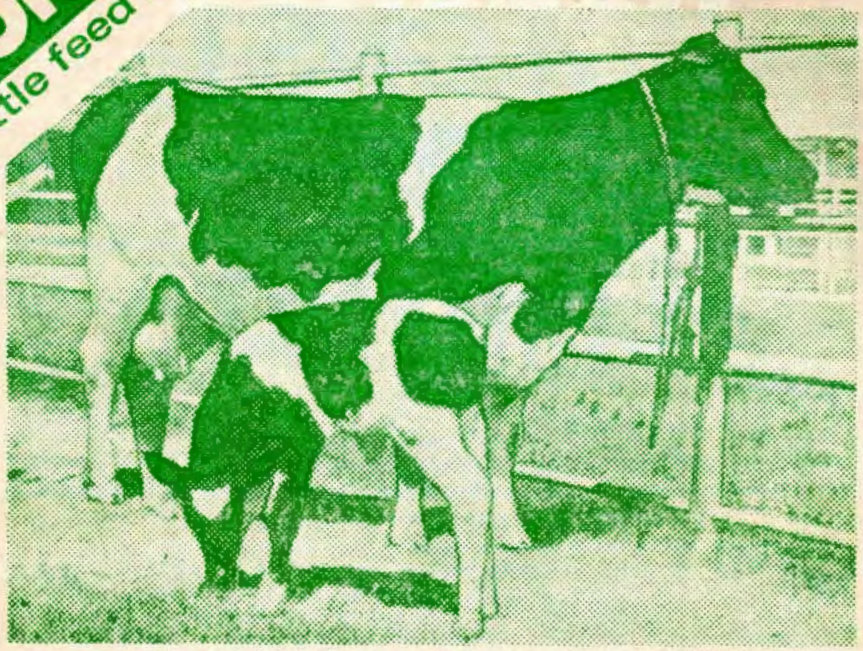

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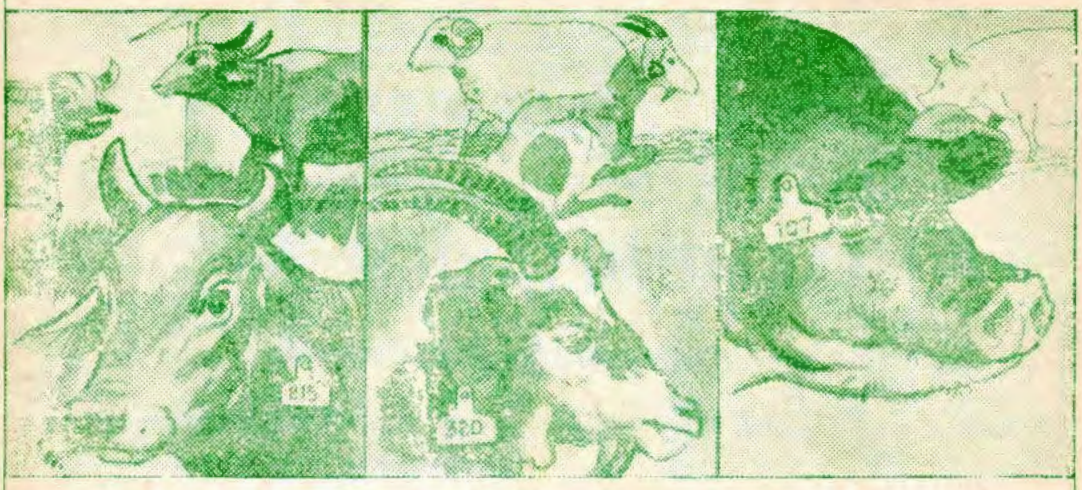

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Vol. 5 No. 1 JUNE 1984

For Particulars Contact

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The Indian Society For The Study Of Animal Reproduction

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THE INDIAN JOURNAL OF
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Vol 5 No. 1 JUNE 1984

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Editorial

AGRICULTURAL BY-PRODUCTS AND LIVE-STOCK FEED

The cost of conventional cattle feeds is increasing day by day and at the same-time there is overall shortage of cattle feeds. The major issue is of finding out of new and cheaper feed resources which are traditionally not used by the cattle owners and thereby minimise the cost of feeding. Such unconventional feeds and by-products can be incorporated in compounded feeds for cattle, buffalo, poultry, swine etc. by compounded feed industry thus replacing the costly conventional feed ingredients such as cereal grains which can be spared for the ever increasing human population. Collection, processing, transportation etc. of new unconventional feeds will also provide employment to the rural and tribal people.

XI th Workshop on I.C.A.R., sponsored *All India Coordinated Research Project on Utilization of Agricultural By-products and Industrial Waste Materials for Evolving Economic Rations for livestock* was held at Gujarat Agricultural University, Anand Campus, Anand—388 001, from 6 to 9 February, 1984. Seventy delegates from I.C.A.R., Head quarters and Institutes, Union Ministry of Agriculture, Project Centres, Agricultural Universities, Compounded Feed Factories and Dairy Cooperatives, participated in the deliberations of the workshop. There are ten Research centres viz. Anand, Bhubaneswar, Gauhati, Hyderabad, Jabalpur, Ludhiana, Pantnagar, Ranchi, Trichur and Urulikanchan. The co-ordinating Research centre is at Jabalpur.

In the key-note address delivered at the workshop by Dr. R. M. Acharya, Deputy Director General (Ani. Science), I.C.A.R., New Delhi, lauded the efforts of animal nutritionists in unearthing newer areas of investigation for the benefit of farming community. He mentioned about very large live-stock population in the country and pointed out that one of the most important impediments in the improvement of their productivity was nutrition. He stressed the need for creating cheaper and balanced rations from the agricultural by-products and waste materials for livestock not only for maintenance purpose, but also to support the ultimate productive functions like growth, reproduction and lactation.

At the inaugural address, Dr. C. B. Shah, Vice-Chancellor, G.A.U. stressed for intensifying research on newer agro-industrial and forest materials for their utilization as livestock feed and emphasized the need for increasing Co-ordinate Research Projects

on Animal Science Subjects. He further gave a brief account of work done at Animal Nutrition Department of Gujarat Agricultural University. The department is engaged in Animal Nutrition Research work since 1942. At Anand, various agro-industrial by-products and forest products were evaluated as animal feeds e.g. Mango seed kernel, *Cassia tora* seeds, *Sal* seed meal, tomato waste, seaweed, *isabgul* byproducts (*Lali* & *Gola*), *Prosopis juliflora* pods, *babul* seed *chuni* etc. The compounded feed industry is utilizing some of these materials like mango seed kernel, *Cassia tora* seeds, *Sal* seed meal, *isabgul* byproducts and *babul* seed *chuni*. The work carried out at these different centres will be useful to farmers and industry.

At present, mango seed kernel, deoiled *sal* seed meal, *Cassia tora* seeds, *guar* meal etc. are being used by compounded feed industry. The workshop emphasized the need for carrying out detailed animal nutrition survey in different regions.

Editorial Board

* Source : Proceeding of the XI workshop on I.C.A.R., sponsored A.I.C.R.P. on utilization of Agricultural By-products and Industrial Waste Materials for Evolving Economic rations for live-stock.

IJAR 5: 1: 1-5 1984

The Lethal Effect of Superovulation on The Embryos

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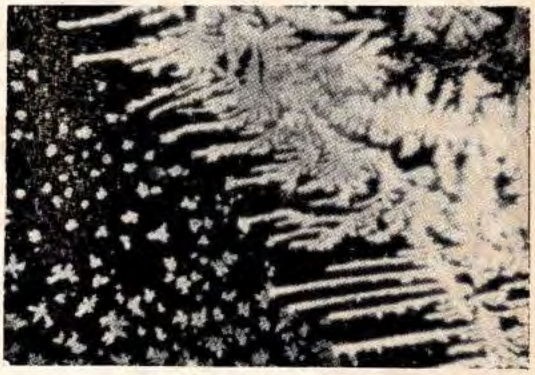
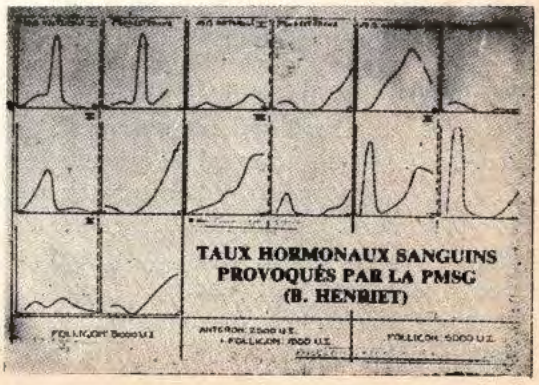
1348. Louvain-la-Neuve. Belgium.

After Dowling's first essay on superovulation with PMSG, Rowson and Lamming established that the eggs obtained were unviable. Experiments with the human species produce similar dissapointing results which are confirmed by Newcomb, Rowson, Trounson, Allen and Stewart. Naturally, the quality of the PMSG has been questioned. The strong individual variations in receptivity to the PMSG have also been blamed. Certainly it is no doubted that some of the subjects in a state of hyposexuality remained refractory to the stimulation. However, their frequency, according to a statistic established over a course of 31 years, is less than 1%. Therefore it is difficult to attribute all of the PMSG failures solely to this ovarian infantilism.





Fortunately, a different path in bringing together three very different types of hormonal studies has been followed. The first is the chemical and microscopic structure of the cervical glair during the oestral cycle. The second concerns ovarian cysts and the third deals with superovulation. A certain similarity between the superovulated cow and the cow carrying ovarian cysts has been found.

Eight cows of the White-Blue Race of middle Belgium were used, after a gynecological examination. Their ages varied from 2-4 years old. The cycles are induced by administration of prostaglandin Pg $F_2\alpha$ (Dinolytic of Upjohn): 5 ml to all subjects. On the ninth and

tenth day after consecutive estrus, an injection of PMSG was given (3000; 4000 or 5000 IU of Folligon from Intervet, or of Anteron from Schering). On the eleventh and twelfth day, another injection of prostaglandin Pg $F_2\alpha$ was given. Artificial insemination was realised either the thirteenth, fourteenth or fifteenth day of consecutive heat. The embryos were collected on the seventh and eighth day following artificial insemination. Blood was collected each day after the PMSG injection until the day of the collection. The hormonal dosage was made by the radioimmunological method. (R.I.A.)

Results

The eight cows gave a good follicular response to the PMSG. This assertion is brought forth by the 17 β oestradiol and progesterone curves and by the rectal examination of the ovaries. The day of the collection, the ovaries attained the size of a potato, carrying several corpora lutea and some unbursted follicles. So, the quality of the PMSG is not at all suspect. These observations on the ovaries and the dosage agree with the characteristics of the glair. The microscopic examination of the glairs constantly shows a proestral type, as evidence the adjoining photographs.

No cow showed the typical signs of heat, shch as overlapping by the congeners or repeated rhythmic movements of the base of the tail. At no moment, was a true phase of heat observed. We could qualify this period of pseudo-heat in the sense that it demonstrates that there is strong folliculin secretion and that there occurs at the same time, a period of luteal loss. There is thus similarity between the cystic ovary and the superovulated ovary. The only difference

resides in the behavior: individuals with cystic ovaries show a nymphomaniac behavior, while our experimental livestock rarely exteriorized their heats. This behaviour may appear to be curious because the hyperstimulation multiplies the rate of 17 β oestradiol by 20 or more, at the moment of heat.

When considering the tops of curves, variations in the responses of each cow can be established. However, the hormonal levels are, in each case, very high, go far beyond the physiological levels, and can be qualified as abnormal. Better yet, if the injected doses are account for, the aspect of the curves cannot be connected to the administered dose. The unsimilar curves are the expression of disordered responses which can be paired with the unpredictable recovery rate.

All of these facts make one believe that all of the follicles are stimulated. In effect, when the PMSG is administered under the skin, it is reabsorbed by the blood and distributed to all of the cells. The follicles, at least those that will be ovulating, respond. Those that do not ovulate are chronologically and histologically too far from the maturation date. Undoubtedly, they will involute the next time so that superovulation is not followed by nymphomania. Those that ovulated had attained an advanced stage, but this does not suggest that they are all at the same stage, especially in a uniparous species. It seems that they divide by level of maturity in a manner such that the more advanced will be the first to ovulate while the less advanced will ovulate last. Thus, the impossibility of finding one precise day for oestrus can be explained by the fact that some unbursted follicles can still be found 7 days after artificial insemination.

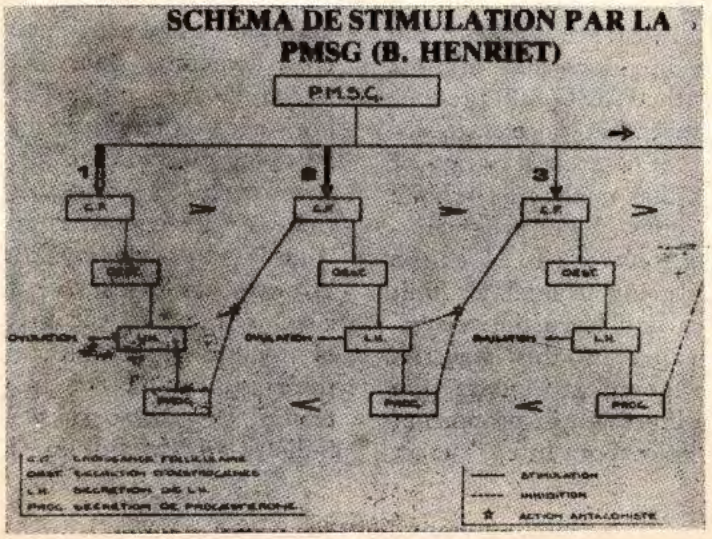
In our experiment, the collections were poor and the quality of the eggs was weak. This agrees entirely with the observations of Schilling who finds that "only small numbers of ova are produced in the majority of cases, that many embryos are not suitable for transfer and that 75% of the variability in the ovarian response remains unexplained". However, the goal of superovulation is to produce viable and numerous embryos.

Through observation of the graphs, it can be said that this is found in the presence of absolutely abnormal doses of hormones. These abnormally high holdings can be paralleled with the chemical studies that were made on tubular fluid during the course of a normal cycle. This fluid is the first way to survey the egg. It follows fluctuations and expresses them by variations in composition, which cause a repercussion on the quality of the egg.

If superovulation is no more than a multiplication of ovulation, only corpora lutea and healthy embryos in a proportional number must he found at the time of harvest.

Hypotheses on the superovulation-mechanism.

First, the PMSG stimulates the follicular growth which is accompanied by



a folliculin discharge. The folliculin stimulates the LH secretion. LH stimulates the progesterone secretion and provokes the ovulation: corpora lutea form. The progesterone of the corpora lutea inhibits the LH secretion. The LH inhibition again allows the action of the PMSG and the cycle starts over again.

This succession of cycles goes on until the PMSG exhaustion and/or when the number of corpora lutea is sufficient to block the ovulation of the latest follicles. Nevertheless, it is to be added that PMSG possesses itself a LH activity; there will therefore be an interference between the LH effect of the PMSG and the anti LH effect of progesterone.

How can the enrollment of this antagonism be imagined? When the PMSG stimulates the growing of the first follicles, it induces a luteinizing secretion too. The progesterone rate still being too weak, the follicular increase ends at ovulation, under the combined effect of LH of PMSG and of LH of hypophysial response at the first oestrogen pick. Then, the first corpora lutea are formed; the progesteronaemia increases and inhibits the LH secretion; the PMSG, still present, and the LH diminution accelerate together a growth of a second series of follicles, the ones that could not ovulate follow the first ones. The progesteronaemia still being too weak to block the follicles, these are going to ovulate, form new corpora lutea and have the progesteronaemia increased in this way. As the follicles are going to burst, the progesteronaemia increased in proportion with the number of corpora lutea, but on top of that it will be stimulated by the LH effect of the PMSG.

At the same time, the PMSG runs low and the number of follicles destined to ovulate reduces. These follicles burst by themselves since they continue to secrete folliculin which stimulates a LH discharge. When the last of the follicles are bursted the progesteronaemia is such that it blocks the ovulation of all others, putting them in a similar state to the cystic follicles of nymphomaniac cows. Here again, we rejoin our hypothesis from the start.

Conclusion

A. The PMSG quality is not implicated anymore.

B. Our hypothesis verifies itself since: we have observed similarities between the superovulated cow and the cow with an ovarian cyst; the cervical glair of one and the other shows a permanent prooestral state. These states, however, differ from one another as proven by their hormonal rate. The collection deficiencies and the bad quality of the embryos can be attributed to the consecutive hormonal rate in superovulation which is very high. The rates of both hormones responds to toxical and teratological doses. This indicates that superovulation is not a simple multiplication of normal ovulation. If the first stimulated oocytes ovulate normally, the last ones are blocked or disturbed by the corpora lutea of the first. The embryos or oocytes of the first are normal, but their quality is compromised in the tromp by the oestrogens of the last follicles which did not burst.

REFERENCES

De Vuyst, A. and al.Etude de la glaire cervicale des bovidés. Zootechnia, IX, 2 (44)-1960.

Dowling, D.F. Journ. Agr. Sci. 39, part 4. 1949.

Edwards, R.G. and Steptoe, P.C. Reimplantation of a human Embryo with subsequent tubal Pregnancy. Lancet 1, 880-882-1976.

Henriet, L. and Vervack, W. Etude de certaines propriétés physiologiques de la glaire cervicale des bovidés. Annales de Médecine Veterinaire, III, 169-176. 1963.

Henriet, L. Etude de quelques propriétes physiques, chimiques, microscopiques et biologiques de la glaire cervicale des bovidés. Bull. Soc. Roy. Belge Gynec. Obst. 34 (5). 1964.

Lopata, A. More studies on the fertilization of human eggs in vitro. Research in Reproduction, 10-1978.

Miller, B.G. and Armstrong, D.T. Effect of superovulatory dose of pregnant mare serum gonadotrophin in ovarian function, serum oestradiol and progesterone levels and embryo development in immature rats. Biol. Reprod., 25, 261-271. 1981.

Newcomb, R. and al. The entry of superovulated eggs into the uterus. Agricultural Research Seminar: Egg Transfer in Cattle. (CEC), EUR 5491, 1-18. 1976.

Rowson, L.E.A. and Lamming G.E. Superovulation and Ovum Transplantation in Cattle. IIth Int. Congre. Phys. Path. of Anim. Reprod. and A.I. 1952.

Saumande, J. Relationships between ovarian stimulation by PMSG and steroid secretion. Current Topics in Veterinary Medicine. Control of Reproduction in the Cow. (CEC), 168-193. 1978.

Schilling, E. The variability in the superovulatory response of donor cows to PMSG and its potential reasons. XVIIth Simposio Int. Zootecnia. Milan. 1982.

Sreenan, J.M. and al. Ovarian responses in relation to endocrine status following PMSG stimulation in the cow. Current Topics in Vet. Med. Control of reprod. in the Cow. (CEC), 144-158. 1978.

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A Heart-Lung-Kidney Like System For the Maturation, Fecundation of Oocytes and Culture of Embryos

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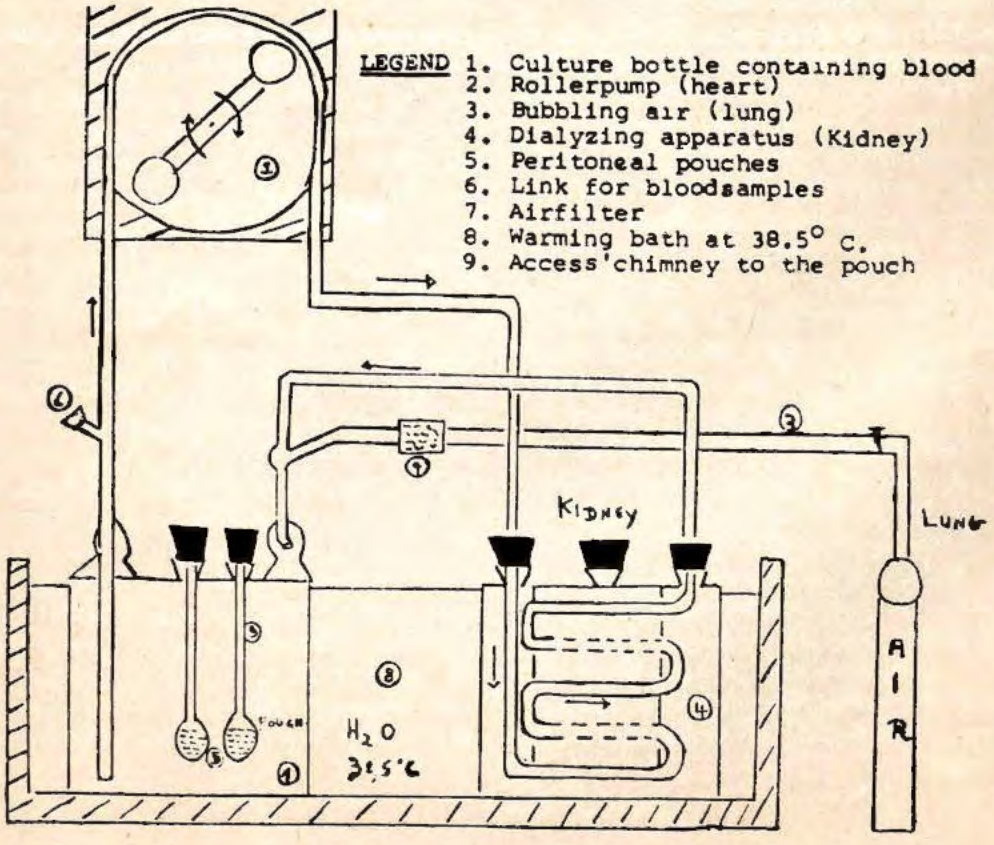
In vitro fertilization has been studied for a long time but it doesn't succeed in all species. In fact, it only succeeds in sheep, rat, mouse, hamster and human species. In 1982 however, Brackett announced the birth of a calf resulting of an in vitro fertilization. In 1983, the second birth was announced in USSR. Those results are too weak to be considered as a successful technique of reproduction. We expose here under the results of some limited experiments.

The engine here presented is built in harmony with the natural conditions of



survey and maturation of the oocytes. First, the embryotron furnishes nutriments, hormones and oxygen to the oocytes. In a second time, it removes the waste products of metabolism. The scheme shows that it is composed of 4 parts: a blood circuit, a dialyzing chamber, an air injection system and a container for the cultured cells. A pump furnishing a pulsion movement like the heart does, moves the blood in the circuit. The blood is defibrinated by churning, oxygenated by bubbling air and depurated by the dialyzing apparatus. The entire system is thermostatized in a warming bath at 38·5°C corresponding to cow's body temperature.

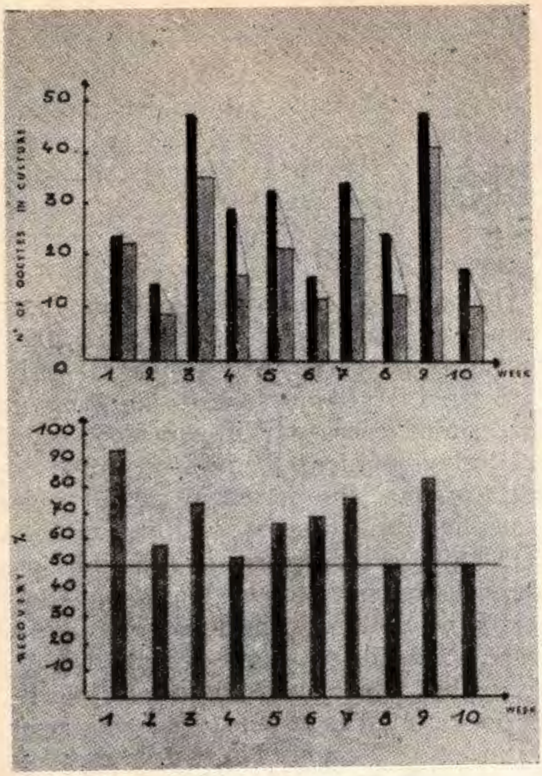
The bovine oocytes are taken by puncture of ovarian follicles from ovaries recovered at the slaughterhouse. In the course of the latest year, an average of thirty oocytes were cultivated each week in a peritoneal pouch containing 2 cc of a chemical defined medium Ham-F-10 selected for his appoint of nutriment. This pouch is fixed to a glass tube and plunged in the blood of the culture phial. The glass tube arises above the blood level, so that it can be used as an access chimney for inserting the sperm and for addition of culture medium or other substances.

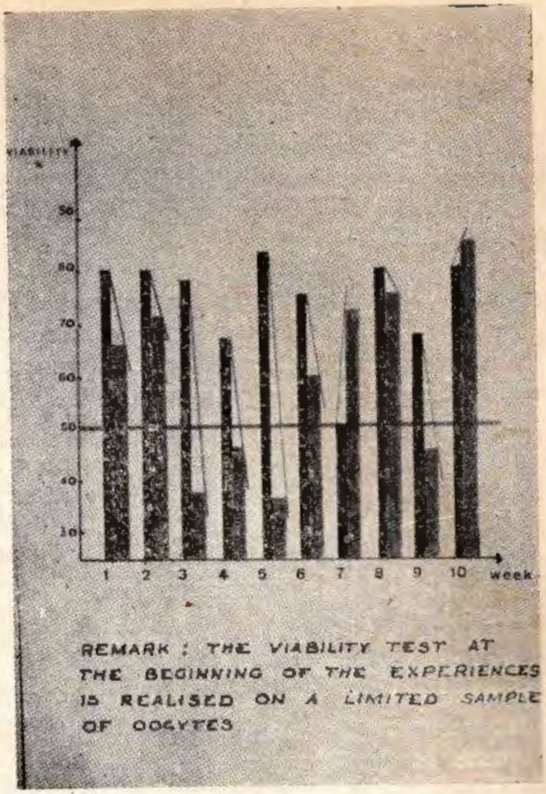
For rat embryos, one situate precisely by vaginal smears in what stadium of cycle rat is. In pro-oestrus stadium, female and male rats are put in presence during night. The presence of spermatozoa in smears next morning is a mean to control whether there was pairing or not. Female rats are sacrified on the third day. The first uterine born, filled up with the Ham-F-10 substance is put in culture just as it is, with the embryos it contains. The second one is washed with a physiological saline solution and the collected embryos (at the morula stadium) are set down in peritoneal pouches of bovines which contain the Ham-F-10 solution. So they are cultivated in the same conditions as bovine oocytes.

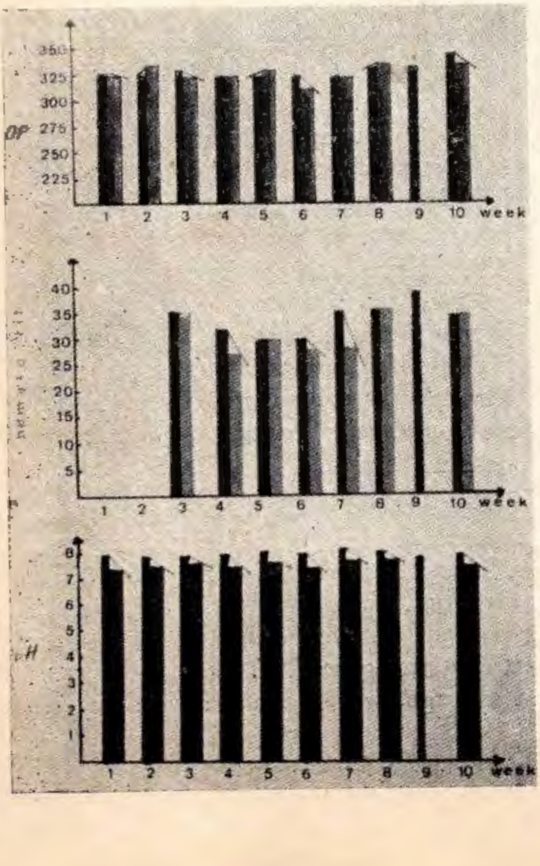
Blood and dialyzing solution are controlled in each experiment: pH, osmotic pressure and hematocrit are measured. Antibiotics and antifungics are added every day to the blood and dialyzing solution. Every day, one tries to reproduce the hormonal climate surrounding the bovine oocytes' maturation, and that is attempted by addition of steroid hormones to the blood and the dialyzing solution. The first day of culture is considered as the second day of cycle and corresponds to an oestrogen ration of 12 Pgr/ml and a progesterone ratio of 2 Ngr/ml. The third day of culture corresponds to theoretical ovulation and 2 inseminations are realised with fresh sperm. The spermatozoa are washed by 2 successive centrifugations at 1500 rpm during 10 minutes. The sperm is then capacitated in a capacitating phial at 38·5°C during 2 hours. Afterwards, one or two drops of this sperm are injected through the access chimney in the peritoneal puch.

The survey of bovine oocytes is controlled by a viability test based on a fluorometric method using fluorescein diacetate. The survey of rat embryos is controlled by comparing their stadium before and after culture.

The recovery rate of the bovine oocytes reaches the average level of 71% and the maximum raises 94%. The viability rate oscillates between 36 and 85%. The maturation figures represent 38% of the recovered female gametes and the fertilization figures 7%. For rat embryos, an average of 67% are found at the end







17β [illegible] AND PROGESTERONE [illegible]

To be compared with the physiological levels:
oestradiol 17β : from [illegible] to [illegible] pg/ml
progesterone : from 0,5 to 7 ng/ml

	1	2	3	4	5	6	7	8	9
I	< 2,5 5,4	48 10,2	41 5,5	256 4	8 1,5	4,1 2,3	<2,5 33,7	/	/
II	< 2,5 11	82,5 1,3	127,5 2,4	9,5 0,15	< 2,5 2,7	3,9 4,8	14 56	< 2,5 177	/
III	7 9,1	52,5 11,4	16,5 0	57,5 11,3	43 34,6	9,25 52,3	/	/	/
IV	< 2,5 40	3 11,8	31,5 6,5	135 0	160 8,21	200 0,3	256 4,7	250 1,9	75 1,4
V	< 2,5 6,3	4,6 10,7	17,25 4,1	2,5 0,3	5,25 1	13,25 5,1	30 26	48 35,6	< 2,5 10,4
VI	2,7 3,5	4,5 3,5	4,6 3,2	30 0,43	52,5 3,7	92,5 4,7	185 7,9	185 11,4	160 17,4
VII	3,6 3,4	5,5 10,1	13,25 7,9	35 0,4	65 2	75 4,3	115 11,8	210 9,8	210 12,8

In each case, the top number expresses the pg of 17β-oestradiol and the bottom number expresses the ngr of progesterone.

Note that the stimulation multiplies the hormone levels by [illegible] more.

Measurements at the beginning of the culture								
Nr of oocytes in culture			Viability (nr of fluorescent oocytes)	pH		osmotic pressure(mosm/l)		hematocrit (%)
denuded	with corona radiata	total		blood	dialysis	blood	dialysis	
20	3	23	4/5	7.84	7.30	327	325	18*
11	3	14	4/5	7.77	7.49	323	336	12*
40	7	47	7/9	7.82	7.40	333	332	35
22	7	29	4/6	7.82	7.39	322	329	32
27	5	32	5/6	7.85	7.39	321	336	29
14	2	16	3/4	7.83	7.34	321	322	29
26	8	34	4/8	7.94	7.32	322	324	35
20	4	24	4/5	7.92	7.41	332	324	35
45	5	48	6/9	7.94	7.48	333	326	38
9	9	18	4/5	7.94	7.48	338	326	34
25	2	27	4/8	7.78	7.39	328	331	29
19	5	24	—	7.77	7.40	327	322	28
31	8	39	2/8	7.88	7.47	333	325	35
53	2	55	5/7	7.92	7.35	337	328	35
46	7	53	4/5	—	—	—	—	—
39	6	45	8/10	7.97	7.38	338	327	34
25	3	28	4/6	7.94	7.43	328	329	36
36	2	38	13/27	7.93	7.43	336	336	36
50	5	55	7/7	7.89	7.31	336	335	38
50	0	50	4/7	7.79	7.30	338	335	38
53	2	55	—	7.78	7.33	333	323	34
35	4	39	5/7	7.76	7.35	333	335	32

Measures at the end of the culture					
Nr of experiment	pH		osmotic pressure		hematocrit
	blood	dialysis	blood	dialysis	
1	7.51	7.32	324	324	17
2	7.49	7.31	333	329	9
3	7.46	7.36	321	330	35
4	7.43	7.30	322	330	27
5	7.58	7.22	330	336	29
6	7.41	7.22	321	321	28
7	7.50	7.25	321	322	28
8	7.42	7.27	334	321	35
9	—	—	—	—	—
10	7.55	7.30	333	326	34
11	7.44	7.22	323	320	28
12	7.46	7.24	321	321	25
13	7.41	7.34	333	328	40
14	7.40	7.27	334	328	40
15	—	—	—	—	—
16	7.53	7,37	334	326	33
17	7.50	7.31	326	329	36
18	7.48	7.28	330	332	36
19	7.45	7.30	335	333	38
20	7.46	7.25	332	333	38
21	7.41	7.27	333	323	33
22	7.51	7.28	329	331	32

Nr of refound oocytes total	with corona radiata	viability	maturation figures	fecundation figures	oocytes' recovery %
22	3	14/21	7	2	94.6
8	2	5/7	3	1	56.8
35	7	12/32	11	2	73.5
16	5	7/15	5	1	52.4
21	5	7/19	8	1	65.1
11	2	6/10	3	1	68.2
27	8	18/25	11	2	75.6
12	3	9/12	4	2	50
42	5	18/39	19	1	84
9	6	6/7	3	1	50
17	2	5/8	8	1	63
10	3	6/8	5	0	41.7
20	5	14/20	9	2	51.2
42	2	19/36	18	3	76.4
44	6	35/44	20	3	83
40	4	27/39	18	5	89
26	3	13/26	11	1	93
32	1	10/17	11	2	84.2
46	4	29/46	18	3	83/6
35	0	18/31	13	0	70
44	1	25/39	15	2	80
31	2	19/31	12	2	79.5
					71%

Viability % at the end	% of maturation figures	% of fecundation figures
65.8	31.8	9
71	37.5	12.5
37.2	31.4	5.6
46.2	31.25	6.25
36.4	38	4.7
60	27.25	9
72	40.75	7.4
75	33.33	16.5
45	45.25	2.3
85.2	33.33	11.1
62.5	47	5.9
75	50	0
70	45	10
52.8	42.9	6.14
79.5	45.5	6.8
69.2	45	12.5
50	42.3	3.8
58.8	34.4	6.25
63	39.1	6.5
58	37.1	0
64	34	4.5
61.3	38	6.5
61.7	38.6	7

RAT EMBRYOS

number of embryos after washing the uterus	Development stadium before culture	Number of embryos at the end of culture	Development stadium at the end of culture
5	morulas	3	blastocysts
7	morulas	3	blastocysts
2	morulas	2	blastocysts

of the culture and that all those 67% have continued their development in vitro. Concerning rat embryos cultivated in isolated uterus, they are all implanted.

Several conclusions can be drawed. The fertilization in vitro failed. The 7% of fertilization figures can't be considered as a success. They are strictly limited to the earliest stages of penetration. Until today we can't affirm that this system could possibly take the place of an in vivo fertilization.

The system used for culture seems to be good. Several years were necessary to work out the apparatus but also to put the final touch to the movements of experimentalists, to the aseptic cares applied to the gathering of oocytes, to the built-up of a peritoneal pouch and the assembling of apparatus. The choking of tubes by clots, for example, thwarted the tests during several weeks. Those failures vanished after the moment two ascertainments were made: first the presence of E. Coli resisting to the combination of penicilline-streptomycine, but sensible at chloramphenicol, and secondly, the lashing of blood which eliminates coagulating substances and makes it possible to dispense with Na-citrate or other anti-coagulating substances. The parameters that were measured in the course of experience, pH, osmotic pressure and hematocrit, allow to verificate the maintain of the initial conditions during each experiment. The measures show a noteworthy invariability from day to day in such a way that the daily samples during the first tests were spread and finally restricted at one sample at the beginning and one at the end of culture.

The first tests only lasted 4 days. Actually, however, the length of the experiences reaches 10 days.

Conclusion

Our principal objectif which was the in vitro fertilization has not been attempted and we attribute this failure to a lack of precision in hormonal balance. On the other hand, maturation of bovine oocytes was easily realised. What's more, embryos survived during several days. The quality of life is that good that embryos cultivated in an isolated uterus took root in it although the blood used in the circuit proceeds from bovines and constitutes an heterologous environment containing substances that thwart transplantations of organs between different species.

So the system could suit as a means of conservation for embryos on short term, if only to make agree hormonal states between donor and receiver. Finally, after been utilised since three years, the artificial heart-lung-kidney like system has proved that it may be considered as a means for culture of all cells.

REFERENCES

Beaudoin, A.R. Embryology and Teratology. The Laboratory Rat, II. Academic Press. 1980:75-101.

Brackett, B.G. and al. Fertilization and early development of cow ova. Biol. Reprod. 23:189-205. 1980.

Brackett, B.G. and al. Pregnancy following cow in vitro fertilization. Biol. Reprod. 24 (suppl. 1):173-190A 1981.

Brackett, B. G. and al. Normal development following in vitro fertilization in the cow. Biol, Reprod. 27: 147-158. 1982

Brackett, B. G. A review of bovine fertilization in vitro. Theriogenology, 19:1-15. 1983.

Fukui, Y. and Sakuma, Y. Maturation of bovine oocytes cultured in vitro: relation to ovarian activity, follicular size and the presence or absence of cumulus cells. Biol. Reprod. 22:669-673. 1980.

Kane, M.T. The effects of pH on culture of one cell rabbit ova to blastocysts in bicarbonate buffered medium. J. Reprod. Fert. 38:477-480. 1974.

Menezo, Y. and al. Attempts at in vitro fertilieation in calf. (CEC) Agricultural Research Seminar-Egg Transfer in Cattle. 1976:189-198.

Mohr, L.R. and Trounson, A.O. The use of fluorescein diacetate to assess embryo viability in the mouse. J. Reprod. Fert. 58:189-196. 1980.

Renard, J.P. and al. Attempts to predict the viability of cattle embryos produced by superovulation. (CEC) Current Topics in Veterinary Medicine. Control of Reproduction in the Cow. 1977:398-417.

Renard, J.P. and al. In vitro uptake of glucose by bovine blastocysts. J. Reprod. Fert. 58:161-164. 1980.

Wright, R.W. Jr. and Bondioli, K.R. Aspects of in vitro fertilization and embryo culture in domestic animals. J. Anim. Sci., 53:702-729. 1981.

IJAR 5: 1: 13-15 1984

Complement Ajoute Au Texte De La Conference:Resultats De Recherches Achevees Entre L'envoi Des Textes Pour Publication Et Le Jour De L'expose Oral (y compris les commentaires)

Why?

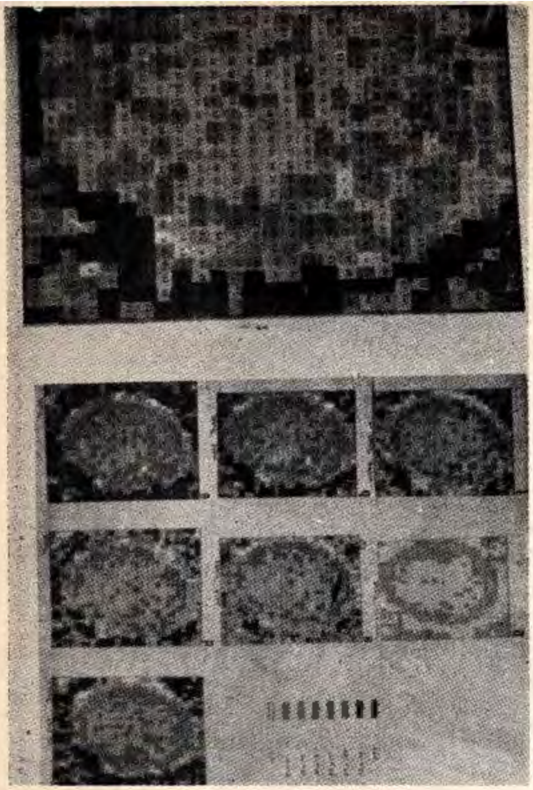
Well, one could answer that we were unable to reproduce the exact Fallopian medium: this one varies at each moment, and it is a funny thing to find and to reproduce a medium who is so inconstant within the period of a full oestral cycle. It is the first cause of failure. Second cause of failure: the oocytes inserted in the pouch are all comers, picked up at the slaughterhouse, at an indefinite stage. A third cause is the quality of the cultured cells, and, here, it may be said that an accurate test should be wellcome. Indeed, the quality of life, the survival capacity of our oocytes was not determined, evaluated, no more this of the embryos.

Well, the superovulation induced by PMSG is well known for the erratic and unforeseeable harvests. An explanation was searched in the hormones levels, in the bovine. It was observed that the 17β Oestradiol and the progesterone levels surpasse the normality and tend to the teratological amounts area, the area of the pharmacologists in their determination of toxicity. Those levels create an abnormal hormonal climate, about which the knowledge is still poor. Think to the morning after pill? What happens with the egg? What happens, the evening after? Well, it could be showed that PMSG doesn't induce a true oestrus. The cow stays in a permanent pro-oestral phase, reminding nymphomania. In fact, I repeat that an accurate, rapid and harmless test should be appreciated. That was established for the sperm, his fertilizing power can be predicted with exactness. But those tests, of which the most are derived from the metabolism, are applied to a mass, to a large number, what should be a non-sense for the egg. Moreover, the compounds and his organels change unceasingly, since the ovogony first cleavage until before and after fertilizaion.

A particular case is this one of the nucleic acids. Baltus and Brachet showed that the Batracian egg accumulates about 5000 thousands more nucleic acids as those wanted for the diploidic amounts. This uncredible reserve is accumulated in view of the nuclei building in the morula, because this morula is unable to synthetise so much DNA. So, one oocyte, egg or young embryo, short of such reserves, has poor survey chances. It is possible to evaluate those amounts of DNA. Well, it was made by mean of UV microspectrophotometry using a Zeiss microscope, Zeiss applying the first results of Caspersson after those of Lison. I have not time enough to explain all the steps of this research. In short, the oocyte was cut in twenty slices, each one was scanned, giving 120 maps of normality. After that, all the disturbances were discarded. The DNA and RNA could so be located and evaluated.

That is applicable to all substances interfering with the microscopic light.

It remains, and that is not a light venture to measure the living egg, before transfer or fertilization and to compare

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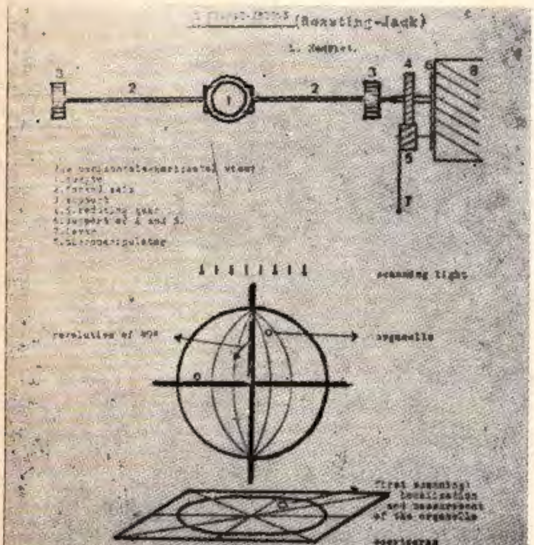
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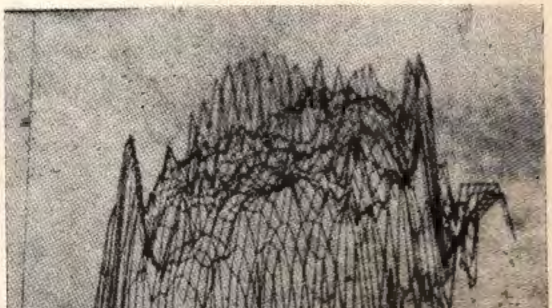

the results with those maps of normality. But, the maps restitute the picture of a plane, of a section through the egg, a killed egg, what is unadmissible, what is forbotten strictly, before transfer.

Well, it is possible to handle the egg under the UV microscope and to pick up him, undamaged out the culture, with the roasting, rotating kitchen-Jack. We did not find the just word for the "Tourne-broche". The rotation of the egg allows the scanning of several planes, giving several maps. Each map must be cleaned out of all the other interfering substances and by successive substractions, the localization and the measurement arise from itself and can be compared to the maps of normality.

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The Effects of Restricted Cobalt Intake on Plasma Profiles of Oestrogens, Progesterone, LH and Corticosteroids in Goats.

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ABSTRACT

Goats were made vit. B_{12} deficient by feeding them cobalt deficient rhodes grass hay for 23 weeks in order to study the effects of the deficiency on plasma sex hormones profiles. Maximal reduction in plasma vit.B_{12} occurred after 8 weeks in parallel with reduction in body weight, Hb and onset of macrocytic normochromic anaemia. Peak plasma progesterone and oestrogens remained comparable to controls for the first cycle, but progesterone rose in the second cycle, before declining sharply. Oestrous LH surge showed an increase to peak values during the third oestrous cycle before declining. Plasma corticosteroids was elevated during the 23 weeks of study. At the end of 23 weeks pituitary LH was less than one third of control values. We conclude that cobalt deficiency causes malnutrition stress which, in turn, leads to elevated LH, Oestrogens, Progesterone and Corticosteroids. The initial elevation of plasma LH causes down—regulation of ovarian LH receptors eventually leading to the atrophy of the gland.

* * *

Ruminants are capable of deriving their "total" vitamin B_{12} from the endogenous synthesis of the vitamin by the ruminal microorganisms (Marston, 1952; Underwood, 1977). They, however, require regular and adequate ruminal cobalt concentrations in order to effect this synthesis (Smith and Marston, 1970; Gombe and Verjee, 1976). A deficiency of either cobalt and/or vitamin B_{12} impairs the conversion of propionate to succinate in carbohydrate metabolism (Cannata *et al.*, 1965; Marston *et al.*, 1972); a form of energy metabolism inefficiency develops. Vitamin B_{12} deficiency is reported to impair reproductive functions leading to cessation of cyclic activity (Marston, 1952; Underwood, 1977). During cobalt/vit.B_{12} deficiency there are various forms of gonadal dysfunctions which are possibly accompanied by impaired secretion of sex hormones. Reduced feed intake has also been associated with increase or decrease in the secretion of sex hormones and gonadal dysfunctions which are possibly accompanied by impaired secretion of sex hormones. Reduced feed intake has also been associated with increase or decrease in the secretion of sex hormones and gonadotropins (Setchell *et* al., 1965; Donaldson *et al.*, 1970; Hill *et al.*, 1970; Gombe and Hansel, 1973; Beal *et al.*, 1978; Imakawa *et al.*, 1983).

Research on the role of the pituitary gland in malnutrition has produced inconsistent results. Atrophy as indicated by both degranulation and decrease in cell size has been reported in humans, dogs, pigs, cattle, sheep and goats

(Lamming, 1966; Jubb and Kennedy, 1970; Heard and Sterwart, 1971; Pimstone, 1976). Contrary results have been reported in guinea pigs (Heard and Sterwart, 1971) rats (Srebnik and Nelson, 1962; Srebnik, 1970); that compensatory increase in size occurs as response to malnutrition. All in all, the above changes are attributed to altered gonadotropin output by the pituitary gland (Gupta and Anand, 1973). The aim of the present study was to correlate the influence of cobalt and vitamin B_{12} deficiency with the dysfunction of the reproductive organs, and to show the relationships between reduced feed utilization and the plasma profiles of sex hormones corticosteroids and gonadotropins.

Materials and Methods

Animals: Ten female East African Short horned goats, 14 to 18 months old, with a weight of 16 to 28 kg were divided randomly into two groups: control (group A) and experimental (group B). Before the experiment all goats were observed through four oestrus cycles in order to ensure that they were cycling normally. All were trained to handling and bleeding for 2·5 months to minimise subsequent inexperiment variability which could be attributed to stress of handling.

Feeding: The experiment lasted 23 weeks. During this period all animals were fed the cobalt deficient diet (less than 0.01 mg per kg/dry matter) containing mainly rhodes grass (*Chloris gayana*) hay. This diet provided 885g total digestible nutrients and 54g digestible protein per goat per day. To prevent cobalt deficiency in control animals (5 goats), this group received Ig cobalt oxide bullets every three weeks.

Sampling: All animals were weighed weekly. Oestrous activity was observed daily and teaser bucks were used to aid oestrus detection. Blood samples were collected at 9.00 a.m. every 2 days during the first month and thereafter at 3-day intervals except during oestrus when bleeding was at 3 hourly intervals. About 6 ml blood was collected from the external jugular vein by venipuncture into universal bottles containing EDTA (2mg). The blood samples were chilled in an ice bath until they were centrifuged at 2500g for 10 min. Plasma was aspirated under sterile conditions and stored at —20°C for future hormonal analysis. At the end of 23 weeks all the goats were killed. Their pituitary glands were weighed and kept at —20°C for LH determination later on.

Analytical Procedures: Red blood cells, packed cell volume, and haemoglobin were determined by the "coulter" counter haemoglobinometer. Vitamin B_{12} was measured by the radioisotopic competitive inhibition kit marketed by New England Nuclear (Massachusetts, USA. Batch NEN 065). All samples were determined in triplicates. The performance characteristic of the kit were 96% recovery, 4% intra-assay variation, and 7.3% inter-assay variation.

Hormone Determination:

The concentrations of progesterone, corticosteroids, total unconjugated oestrogens and LH were evaluated by specific radioimmunoassays as described by Mgongo *et al.*, (1983). Tritiated steroids (6, 7^3 H oestradiol 58 Ci/mmol Batch 43 TRK 125 and 6, 7,^{3} H progesterone 49 Ci/mmol Batch 14 TRK 341) were obtained from New England Nuclear; unlabelled steroid from Steraloids Inc., (oestradiol S*H*52*H*5 and progesterone S*H*25*H*2) from Guy E. Abrahams, Cali-

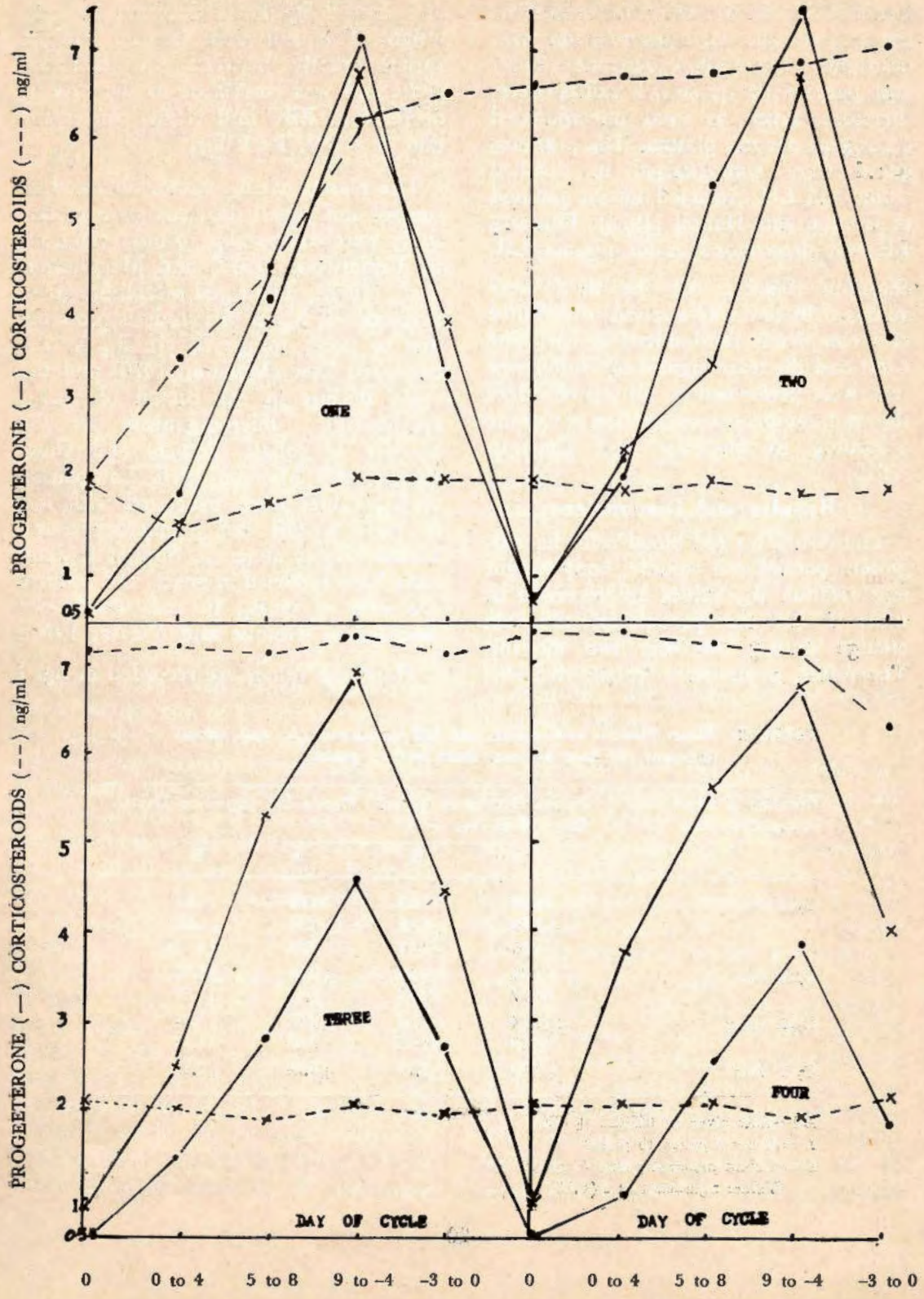
TABLE 1: Characterization of normal and cobalt deficient goats. Changes in RBC, HB, PCV, Body weight, Vit B_{12} and Pituitary gland values in normal (A:n=5) and cobalt deficient goats (B:n=5)

Duration of Experiment in weeks	RBC		Hb		PCV		Body weight (kg)		Vit. B_{12} (pg/ml)		Pituitary gland**	
	A	B	A	B	A	B	A	B	A	B		
I	11.49 ±0.66	12.38 ±0.09	8.58 ±0.52	8.50 ±0.41	26.40 ±2.20	30.80 ±1.72	18.80 ±0.78	20.06 ±1.38	462 ±35	675 ±94	Weight (mg) 295.50*a* ±25.05	402.00 ±54.71
8	11.25 ±0.77	11.65 ±0.45	8.56 ±0.37	7.72 ±0.56	26.40 ±2.38	29.80 ±0.97	18.20 ±0.58	18.60 ±1.44	422*a* ±68	184 ±39	*LR concentration (ug/g)*	
15	11.09*a* ±0.63	8.71 ±0.17	8.56*a* ±0.46	6.55 ±0.46	24.88 ±2.79	19.10*a* ±2.05	19.10*a* ±0.84	14.40 ±0.51	574 ±49	155 ±14	5.77*a* ±1.44	144 ±0.26
											LH concentration (ug/g)	
23	11.72*a* ±0.52	8.12 ±0.10	8.94*a* ±0.68	6.00 ±0.48	25.80 ±2.56	23.38 ±1.57	19.40*a* ±1.25	13.60 ±0.51	480*a* ±90	128 ±10	101.18*a* ±11.40	46.71 ±4.22

NB A. All values given as means (±sem). A = Control group (5 goats)

B. Cobalt deficient goats (5 animal). Superscript "a" signifies P< 0.05
Pituitary gland values are thos at 23 weeks.
Measurements in RBC (10^{12}/litre); Hb (g/dl) and PCV (litres/litre)

FIG. I. MEAN PLASMA PROGESTERONE (—) AND CORTICOSTEROIDS (---) IN CONTROL (x) AND COBALT DEFICIENT (.) GOATS IN CYCLE 1, 2, 3 AND 4



fornia, USA. In the assays for oestrogens the antisera showed cross reaction with oestradiol 17α (40%) oestrone (35%) and oestrial (8%), so all values were, therefore, given as total unconjugated oestrogens per ml. plasma. The pituitary gland were homogenized in distilled water and LH extracted by the method of Gombe and Hansel (1973). Pituitary LH was determined as for plasma LH.

Statistical Analysis: All the data were analysed by one way analysis of variance of a completely randomized design. The t-test and the least significant difference tests were performed on all sets of values showing significance on analysis of variance according to Snedecor and Cochran (1975).

Results and Discussion

The results for red blood cells, haemoglobin, packed cell volume, body weight and vitamin B_{12} values are recorded in table I. All the parameters did not change during the first two months. Thereafter, goats on a cobalt deficient diet showed significant decreases in body weight. The individual loss in weight varied greatly between the deficient goats and was correlated to the rate of decrease in RBC and vit.B_{12} concentration ($r = 0{\cdot}9$; $P<0{\cdot}05$).

The plasma vit.B_{12} values obtained in control and cobalt deficient goats of this study were comparable to those reported by Underwood (1977) and Macpherson *et al.*, (1976). The rapid establishment of avitaminosis B_{12} in this study is probably due to poor storage of vit. B_{12} in the ruminant liver (Marston, 1970) and the rapid decline in the rumen of vit.B_{12} synthesizing microorganisms in the absence of cobalt (Somers and Gawthorne, 1969; Marston, 1970). Low plasma vit.B_{12} values were associated with loss in body weight and a macrocytic and normochromic anaemia. Similar findings have been reported in goats, and confirm the role of vit.B_{12} in red blood cell production (Gombe and Verjee, 1976).

Hormone values are recorded in fig. I

TABLE 2: Mean Plasma oestrogens, and LH in normal (A) and cobalt deficient (B) East African short horned goats.

Duration of Experiment in oestrous cycles	Plasma Oestrogens (pg/ml Day − 1 to Day + 1)		Plasma LH (ng/ml on Day 0)	
	A	B	A	B
Cycle one	64.18 ±6.79	71.07 ±5.68	10.00 ±0.44	14.65 ±1.36
Cycle two	75.86 ±2.34	70.94 ±8.46	9.14 ±0.66	14.51 ±1.68
Cycle three	66.77 ±10.84	58.33 ±7.68	8.45 ±0.63	17.60 +3.96
Cycle four	78.69 ±4.38	44.65 ±2.21	9.06 ±0.29	11.13 ±2.15

All values given as MEAN ± SEM
A = Control group (5 goats)
B = Cobalt deficient group (5 goats)
Values significant at $P<0.05$

& Table 2. Plasma progesterone in control goats was 0·6 ±0·09ng/ml during oestrus and reached maximal values between Day+9 and Day − 4 of the oestrous cycle. Plasma oestrogens followed a similar pattern with maximal values on Day − 1 to Day+1 of the oestrous cycle.

In cobalt deficient goats, plasma oestrogens and progesterone values were similar to those for control animals in the first cycle. In the second cycle, however, peak plasma progesterone (9.83± 0.87 ng/ml) were higher than controls (6.74±0.59 ng/ml <0.05). Peak plasma oestrogens were unchanged in this cycle. In the third cycle, peak plasma progesterone were significantly lower than controls. Similarly, peak plasma oestrogens (58.33 ±7.68 pg/ml) were below those of controls (63.77±10.84 pg/ml). These observations were accentuated in the fourth cycle.

Peak plasma LH values of deficient goats were higher in cycles 2 and 3, and by the fourth cycle the LH values of deficient goats had fallen to control levels. At the end of 23 weeks the pituitary glands of deficient goats (402±52 mg) were significantly heavier than those of controls 295±25 $P<0.01$). But this increase in weight was concomitant with a decline of total LH (46.71±4.22<ug Vs 101.18±11.40 ug for controls, $P<0.01$) and pituitary LH concentration (2.34 ±0.65 ug/g protein Vs 5.77±1.44 ug/g protein for controls, $P<0.01$). The decline in pituitary LH was not dependent on body weight changes nor on pituitary gland weight ($r^2=0.05$ and 0.01, respectively). It should be emphasized that even though the pituitary content and concentration of LH was reduced at the end of 23 weeks, the pituitary weights of deficient goats were significantly increased above those of controls, all indicating increase in pituitary gland activity.

Work in sheep and cows (Cumming *et al.*, 1971; Beal *et al.*, 1978) and in rats (Findlay and Cumming, 1976; Nakanishi *et al.*, 1976) showed that initial increases in LH were found in both the pituitary gland and plasma, and plasma LH declined with the duration of the malnutrition. Changes in hypothalamic—LH —releasing hormone corresponded with but preceeded changes in pituitary LH. Lamming (1966) and Lamond (1970) suggested that the decrease in pituitary LH was not caused by the type of food restriction.

The present results of this study are consistent with the above reports. Other workers, however, have reported of no change in pituitary gland activity (Heard and Sterwart, 1971). Ibrahim and Howland (1972), Howland (1972) obtained higher pituitary LH concentrations in starved ovariectomized rats than in those ovariectomized but on normal diets indicating that the effects of malnutrition are independent of ovarian feedback effects.

Our results show that cobalt deficiency increases plasma LH, progesterone and oestrogens during the early stages with subsequent decline in all the hormones as deficiency continues. Little or no change was noted in basal values. Similar hormonal profiles have been reported following energy or protein restriction in various species (Donaldson *et al.*, 1970; Cumming *et al.*, 1971; Gombe and Hansel, 1973; Folman *et al.*, 1983). It will thus be seen that there is remakable similarity in hormone profiles following cobalt/vit. B_{12} deficiency and restricted food intake. The concomitant increase and sustained levels of corticosteroids during the 23 weeks in cobalt deficient goats, suggested

an increased adrenal cortex activity and ACTH output, even though this was not measured directly. It would seem, therefore, from the hormonal profiles, that there was a generalized stress effect (Matteri and Moberg, 1982; Chesworth and Easdon, 1983). Stress has been shown to stimulate and then inhibit release of trophic hormones from the pituitary gland (Ganong, 1977).

In conclusion we postulate that cobalt/vit.B_{12} deficiency, by impairing carbohydrate digestion and metabolism in the ruminant, leads to chronic malnutrition. The malnutrition stress, in turn, causes an initial over-secretion of LH (and other trophic hormones including ACTH). The ovaries respond to the increased plasma LH by increased steroid output before down-regulation of LH receptors lead to ovarian atrophy.

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REFERENCES

Beal, W.E., R.E. Short., R.B. Staigmiller., R.B. Bellows., C.C. Kaltenbach and J.G. Dunn (1978). *J. Anim. Sci.* 46:181.
Cannata, J.J.B., A. Foceri., R. Mazumder., R.C. Warner and S. Ochoa (1965). *J. Biol. Chem.* 240: 3249.
Chesworth, J.M. and M.P. Easdon (1983) *J. Steroid Biochem* 19: 715.
Cumming, I.A., B.J. Mole, M.A. de'Blockey., C.G., Winfield and J.R. Goding (1971) . *Ji Reprod. Fert.* 24: 146.
Donaldson. L.E., J.M. Bassett and G.D. Thorburn (1970). *J. Endocr.* 48: 599.
Findlay, J.K. and I.A. Cumming (1976). *Biol. Reprod.* 15:355.
Folman, Y., M. Rosenberg., I. Ascarelli., M. Kaim and Z. Herz (1983). *J. Steroid Biochem.* 19: 863.
Ganong, F.W. (1977)., In "*Reproduction in Domestic Animals.* 3rd)Edt. Academic Press, New York.
Gombe, S. and W. Hansel (1973). *J. Anim. Sci.* 37: 728.
Gombe, S. and Z.H. Verjee (1976). *Inter. J. Vit. Nutr. Res.* 46: 165.
Gupta, S.R. and B.K. Anand (1973) *Indian J. Phys. Pharm.* 17: 334.
Heard, C.R.C. and R.J.C. Stewart (1971) *Hormone* 2; 40.
Hill, J.R., D.R. Lamond., D.K. Henricks., J.P. Dickey and B.D. Niswender (1970). *Biol. Reprod.* 2: 78.
Howland, B.E. (1972). *J. Anim. Sci.* 34: 445.
Ibrahim, E.A., and B.E. Howland (1972). *Canadian J. Phys. Pharm.* 50: 768.
Imakawa, K., R.J. Kittok and J.E. Kinder (1983) *J. Anim. Scil* 56: 454.
Jubb, K.V.F. and P.G. Kennedy (1970). Ist Edt. Academic Press.
Lamming, G.E. (1966). *Nutr. Abstr. Rev.* 36: I.
Lamond, D.R. (1970). *Anim. Breed. Abstr.* 38: 359.
Macpherson, A., F.E. Moon and R.C. Voss (1976) *Brit. Vet. J.* 132: 294.
Matteri, R.L. and G.P. Moberg (1982). *J. Endocr.* 92: 141.
Marston H.R. (1952). *Phys. Ret.* 32: 66.
Marston H.R. (1970). *Brit. J. Nutr.* 24: 615.
Marston, H.R., H. Allen and R.M. Smith (1972) *Brit. J. Nutr.* 27: 147.
Mgongo, F.O.K., S. Gombe and J.S. Ogaa (1983). *Indian J. Anim. Reprod.* 4: I.
Nakanishi, Y., J. Mori and H. Nagasawa (1976). *J. Endocr.* 69: 329.
Pimstone, B. (1976) *Clin. Endocr.* 5: 79.
Setchell, B.P., G.M.H. Waites and H.R. Lindner (1965). *J. Repr. Fert.* 9: 149.
Smith, R.M. and H.R. Marston (1970). *Brit. J. Nutr.* 24: 879.
Snedecor, G.W. and W.G. Cochran (1975) 6th Edt. Oxford and IBH Publish. Co. Calcutta.
Somers, M. and J.M. Gawthorne (1969). *Austr. J. Expt. Biol. Med. Sci.* 47: 227.
Srebnik, H.H. and M.M. Nelson (1962). *Endocr.* 70: 723.
Srebnik, H.H. (1970) *Biol. Reprod.* 3: 96.
Underwood, E.J. (1977). 4th Edt. Academic Press, New York.

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Studies On Antepartum Prolapse Of Vagina In Buffaloes II Evaluation of Different Treatments.

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ABSTRACT

The study was conducted in 43 animals. Epidural anaesthesia was found to be more effective as compared to the pudic nerve block for prevention of straining. Out of the three methods used for the retention of the prolapsed mass namely Buhner's technique, Flessa sutures technique and AG-Tek profix button, the first method was found to be quite effective in retaining the prolapsed mass. The problems associated with the second method were that of inadequate retention of the prolapsed mass and subsequently the vulvar tearing in those animals in which straining was not checked. The major problem associated with the last method was that of formation of fistulous tract in the croup region which served as a source of infection.

As far the use of various medicines in treating antepartum vaginal prolapse is concerned, it was observed that the drugs containing calcium were effective in mild degree of vaginal prolapse cases, but in severe cases of vaginal prolapse, calcium therapy must be supplemented with either of the retention techniques. Calcium and progesterone therapy has no better effects as compared to the treatment with calcium alone.

* * *

Prolapse of vagina is common in buffaloes. Various medicinal and surgical treatments have been adopted. The most common are the use of calcium (Simkiss, 1968, Mannari 1971, Misra 1976, Pandit 1978) and progesterone (Gibbons 1962, Roberts 1971, Pandit 1978) either alone or in combination. In those cases which do not show any response to those drugs, medicinal treatment is coupled with surgical procedures such as either the use of Buhner sutures (Buhner 1958 and Bierschwal 1971) or the prolapse mass is retained by Flessa suture technique (Roberts 1971) and lately the methods involving internal fixation of vaginal wall has been tried (Hentschel 1961, Johanson 1968 and Norton 1969). Although efficacy of various individual treatments had been reported by individual authors (Buhner 1958, Hentschel 1961, Gibbons 1962 and Simkiss 1968) but we have not come across any report giving some conclusive results regarding the efficacy of various treatments. So in the present study both medicinal as well as surgical approach was followed to find out the most effective treatment for vaginal prolapse.

Materials and Methods

The present study was conducted in 43 buffaloes which were brought to the veterinary clinics HAU, Hissar for the treatment of vaginal prolapse.

In order to study the response of anaesthesia in straining reflex the animals

were divided into two groups. Group I consisted of 28 buffaloes and in these animals epidural anaesthesia was tried where as group II comprised of the buffaloes in which pudic nerve block was tried as a measure to prevent straining. The efficacy of various retention sutures was studied by dividing the animals randomly into three groups. These groups consisted of nine, six and eight animals; and Buhner, Flessa and AG-TEK profix button were tried respectively in these animals (Fig 1,2 and 3). In order to study the response of calcium therapy alone and calcium alongwith progesterone the animals were divided into two groups I and II consisted of 16 and 6 animals respectively (Table III).

The schedule of injecting Mifex* was 150 ml. intravenously on first day followed by 100 ml. Subsequently 150 ml. was repeated on alternate days for 15 days. Thereafter 150 ml. was given at three day interval. Progesterone treatment wa. given as 250 mg. Uniprogestin** repeated at five day intervals till approximately ten days before parturition.

TABLE 1 Comparative efficiency of the pudic nerve block and epidural anaesthesia in antepartum prolapse of vagina.

Sr. No.	Number of animals	Pudic Nerve Block	Epidural Anaesthesia
1.	Number of animals	15	28
	Average quantity used (ml)	76.3 (70.86)	5.6
2.	Average time required for cutaneous desenstization (Minute)	11.6 (8.15)	5.6 (3.7)
3.	Effect of Anaesthesia		
	a) Complete	11 Animals	28 Animals
	Partial	4 animals	
4.	Average duration of anessthesia (Minute)	149.5 (105-185)	68.3 (45.65)

Figures in parenthesis indicate the range.

TABLE 2 Relative efficacy of the different types of retention sutures employed in buffaloes with antepartum prolapse of vagina.

Number of animals	Buhner's	Flessa	AG-TEK Profix Button
Number of animals	9	8	6
Duration for which remained i			
in situ (Days) a. Minimum	2	1	8
b. Maximum	60	14	30
Effective retention of the prolapse mass (% animals)	88.9	87.5	66.67
Infection (% animals)	11.1	12.5	33.3
Foetal Delivery			
a. Normal	77.6	87.5	100
b. Assisted	22.2	12.5	—

* May and Baker (India) Ltd. Bombay ** Unichem Laboratories Ltd. Bombay

TABLE 3 Comparative efficacy of progesterone and Mifex therapy in antepartum prolapse of vagina in buffaloes.

Sr. No. Group	Number of animals	Duration of treatment Minimum Days	Maximum Days	Efficacy No.	%
1. Progesterone and Mifex	6	12	40	3	50.00
2. Mifex	16	1	30	13	81.25

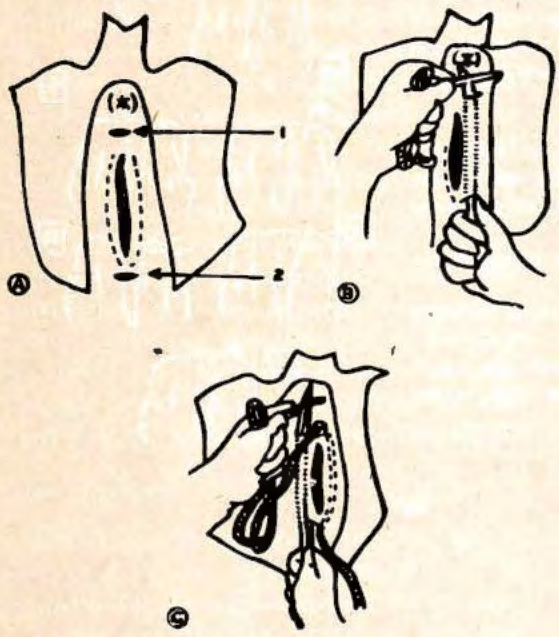


Fig. 1. Line diagram of the Buhner's Technique.

A. Site of skin incisions
   1) Midway between the anus and the dorsal vulvar commissure.
   2) Below the ventral vulvar commisure.

B. Insertion of Buhner's needle through the lower incision.

C. Threading of the needle with the synthetic tape.

Fig. 2. Flessa sutures in situ in a buffalo.

Fig. 3A. Line diagram showing the technique of AG-TEK profix button.

1. Plastic Trocar
2. Plastic Button
3. Stainless Steel Pin
4. Plastic Washer
5. Hitch Pin Cotter

[3] Animal under epidural anesthesia.

[4] Clean up, reduce and replace vagina.

[5] Plastic trocar through 3" plastic button so that the head of the trocar seats in countersunk hole in button.

[6] Stainless steel pin (blunt end first) into centre hole of plastic trocar.

[7] Entering the vagina, insert tip of stainless steel pin through the replaced vaginal wall (right of left), sacro- sciatic ligament, biceps femoris and skin coming out at a point about 6" posterior to the tuber coxae and within 3 to 4 inches of the midline. (An out skin incision will assist penetration of the pin and trocar through the skin.)

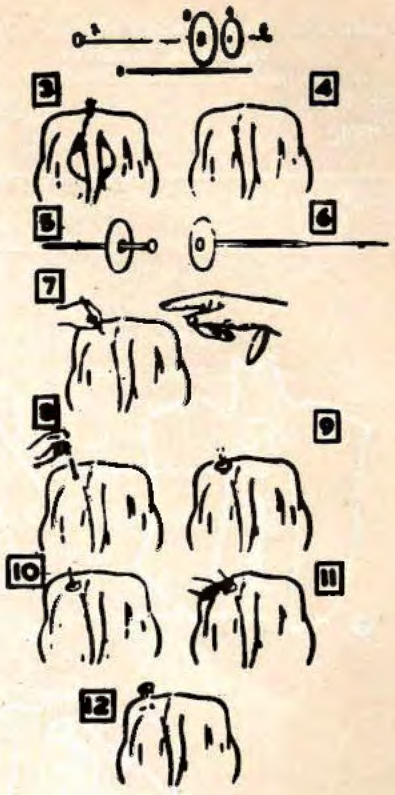
[8] Remove stainless steel pin from plastic trocar.

[9] Holding the plastic button a finger's width from the inner vaginal wall, place the plastic washer over the end of the trocar and push down until vaginal wall is held firmly against the sacrosciatic ligament.

[10] Insert hitch pin cotter through the hole in the plastic trocar closest to the top of the washer.

[11] Cut off the plastic trocar(s) at a point one hole above the hitch pin cotter.

[12] Seven to ten days after calving remove the hitch pin cotter and 2"plastic washer and withdraw the torcar and button from the vagina.



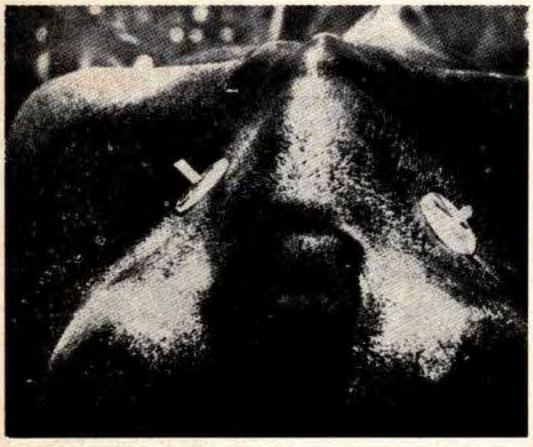


Fig. 3B. Photograph showing the AG-TEK profix buttons in situe in a buffalo.

Results

The efficacy of pudic nerve block and epidural anaesthesia in antepartum prolapse of vagina is shown in table 1.

The comparative efficacy of the different treatments employed are presented in table II and III.

Buhner's technique

The minimum and maximum duration for which these sutures remained in situ was two and sixty days respectively. Out of nine animals treated by this method the prolapsed mass was retained successfully in eight animals. In one animal the sutures gave way and simultaneously developed infection of the suture line. The suture were removed in all the animals before parturition and the parturition in seven animals was normal where as in the remaining two animals the delivery of the calf had to be assisted because of the lateral deviation of the head and the neck-resulting into dystocia.

Flessa technique

The sutures remained in situ for a minimum of one day and maximum of 14 days. Of the eight animals the prolapsed mass was effectively retained in seven animals. In one animal, in which sutures were not effective, there was tearing of the vulva and a mucopurulent discharge from the vagina. The foetal delivery was normal in seven animals, whereas in the other animal a dead foetus was removed by caesarean section.

AG-TEK profix button technique

The buttons remained in situ for a minimum of eight and a maximum of thirty days. Effective retention was achieved in four animals and in the remaining two animals the vaginal mass could be observed gaping at vulvar orifice as and when the animal strained. In two animals which had execessive straining during the period of study fistulous tracts were formed by the plastic trocar, which turned septic with purulent foul discharge. The foetal delivery, however, was normal in all the cows. In one animal the plastic trocar, at the time of removal was found to be broken.

Mifex therapy

Out of the sixteen animals in this group the treatment was effective in thirteen animals. The remaining three animals, which did not respond to this treatment subsequently developed prolapse of more severe nature. These animals were treated with other methods of retention sutures. The treatment was continued in the affected animals for a minimum of one and a maximum of thirty days.

Mifex and progesterone therapy

The treatment with progesterone and Mifex was successful only in three of the six animals in which it was tried. In other three animals which did not respond to this treatment, retention sutures were applied. The minimum and the maximum number of days for which the treatment was continued was twelve and forty days respectively.

Discussion

In the present studies, the anaesthetic effect as judged by cutaneou. desensitization was noticed after an average of 4.6 minutes with epidural anaesthesia. The anaesthetic effect was effect in completely abolishing straining, thereby facilitating the replacement of the vaginal mass. Similar observations were also made by Singh and Johari 1973 and Narasimhan et. al. 1975, in buffaloes. The dose

required for the epidural anaesthesia was less as compared to pudendal block. However, the anaesthestic effect lasted longer with pudendal block, although the induction was delayed and complete desensitization was achieved in only 11 of the 15 animals. It is possible that the failure of adequate desensitization in four animals may be because of incomplete blockage of the middle haemorrhoidal nerve.

A comparison of the two method suggest that epidural anaesthesia has an edge over the pudendal nerve block. In that the onset of desensitization is faster, the anaesthestic effect is longer and the dose required is smaller. It is particularly advantageous in field conditions as the method is simple and easy to perform with little untoward effect.

Retention sutures

Among the three methods employed, namely Buhner's sutures, Flessa technique and AG-TEK button technique, the first two seem to have an advantage over the third. The Buhner's sutures were effective in eight of the nine animals in retaining the prolapsed mass. The foetal delivery was normal in seven out of the nine animals and in the remaining two animals although the delivery had to be assisted, it had nothing to do with the sutures as the foetus were in abnormal posture. The use of Buhner's retention technique has also been successfully employed by Roberts 1971 and Novazzi 1971.

Similarly, the Flessa technique was effective in seven of the eight animals in retaining the vaginal mass. The use of Flessa technique as a line of treatment for vaginal prolapse has been recommended by Pearson 1975 and Walker and Vaughen 1980. However, the Buhner's technique seems to be preferable over the Flessa technique because of it's easiness, simplicity and the sutures can remain in situ over long period without any untoward effects and can be easily removed or released at parturition.

The AG-TEK profix button technique was effective in four of the six animals employed for the retention of vaginal mass. Two animals developed fistulous tracts along the plastic trocar with the vaginal mass being periodically seen at the vulvar lips when the animals strained. The fistulous tracts turned septic with foul odour. The development of these fistulous tracts seems to be a definite disadvantage with the use of this method for the control of vaginal prolapse.

Effect of calcium alone and in combination with progesterone

In the present study the use of Mifex, calcium borogluconate found to be effective in 13 of the 16 animals for the control of vaginal prolapse. The remaining three animals which failed to respond were subsequently treated with one of the retention suture techniques. It was further observed that the treatment with calcium was particularly effective in mild or initial stages of vaginal prolapse. The use of calcium as a line of treatment for the control of vaginal prolpase has also been reported by Chavance, 1944, Ryedell 1954 and Mannari 1971. It is also stated that colloidal calcium preparations act as a general tonic in cases with vaginal prolapse, where there is neuromuscular debility (Mannari, 1971). It also increases the muscular tone of the smooth muscles of the genitalia, (SimKiss, 1968). It also acts by sensitizing the tissue to the action of various hormones (Moddie 1965). Thus the use of calcium

particularly in mild cases of vaginal prolapse seems to be of advantage.

Burch, 1953 and Gibbons 1962 were of the opinion that the progesterone could be used as a preventive or therapeutic agent in cases of antepartum vaginal prolapse in cattle. The findings in the present study do not support their contention. Similar observations have also been made by Roberts, (1971). In contrast, Pandit, (1978) stated that progesterone therapy was effective in animals with antepartum vaginal prolapse.

A comparison of the different techniques indicate that no single method is completely effective, as each method has its own advantage and disadvantage. However, the results of the present study indicate that the parental administration of Mifex (calcium borogluconate) along with the use of Buhner's or Flessa retention sutures were considerably effective in the treatment of vaginal prolapse.

REFERENCES

Bierschwal, C.J. and De Bois, C.H.W. (1971) The Buhner method for the control of chronic vaginal prolapse in the cow. Vet. Med. and Small Anim. Clinician. **66**(3): 232:-236.

Buckaert. J.H. Oyaert, W. Wijverkens, H. and Vens Marihaeghe, E. (1956) Prolapse of the vagina in cows. Vlaam. diergeneesk Tijdschr. **25**: 119-132 (Vet. Bull 27: 1610)

Buhner, E. (1958) Simple surgical treatment of uterine and vaginal prolapse (Translated from German) Tierztl. Umasch. **13**: 183-188.

Burch, G.R. (1953) Progesterone for prolaspe of the vagina J. Am. Vet. med. Ass **122**: 365.

Gibbons, W.J. (1962) Pinning for vaginal prolapse Mod vet. Pract. **43**(6): 82.

Hentschl. A.F. (1961) The button technique for correction of prolapse of vagina in cattle J. Am. vet. med. Ass **139** (12): 1319-1320.

Johanson, R.D. (1968) Repair of prolapsed vagina in the cow. Vet. Med and Small Anim. Clin. **63**: 252-256.

Mannari, M.N. (1971) Prolapse of vagina in buffaloes. Guj. Vet. **5**: 139-144.

Misra, S.S. (1976) Observation on the theraputic management of utero vaginal prolapse in bovines. U.P. Vet. J. **4**: 55-61.

Moddie, E.W. (1965) Modern trends in animals health and husbandry of hypocalcaemia and hypomagnesemia. Br. Vet. J. **121**: 338-349.

Norton, E.S. (1969) External fixation of the vaginal prolapse. J. Am. vet. med. Ass. **140**: 1179-1181.

Novazzi, G. (1971) Treattmento dal prolapse of vaginal cervicle cormieodella bovine mediante to texils de Buhner (Treatment of chronic vagina cervical prolapse in cow by Buhner technique) Atti della Secieta Haliana di Baniatria **3**: 311-315 (Vet. Bull **53**: 968).

Pandit, R.K. (1978) Studies on vaginal and uterine prolapse of buffaloes Ph.D. Disseration Punjab Agricultural University, Ludhiana.

Pearson, H. (1975) Thr treatment of vaginal prolapse in the cow. Vet. Annual **5**: 54-56.

Roberts, S.J. (1971) Veterinary Obstetrics and Genital diseases (Therogenology) 2nd ed. p. 189-196. Scientific Book Agency, Calcutta.

Raydell, R.D. (1954) New treatment for bovine ractal vaginal and uterine prolapse. J. Am. vet. med. Ass **125**: 226-227.

Singh, G.D. and Johari, M.P. (1973) Prolapse of uterus and vagina in buffaloes, U.P. vet. J. **1**: 96-99.

Simkiss, J. (1968) Calcium in reproductive Physiology. A comparative study of the Vetebrates. Chapman and Hall, London.

Walker, D.F. and Vanghan, J.T. (1980) Bovine and equine urogenital surgery Ist ed. p. 75-79 Loa and Febiger, Philadelphia, USA.

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Gynaeco-Clinical Studies On Post-Partun Reproductive Disorders In Buffaloes

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ABSTRACT

Post-partum reproductive disorders were studied in 119 buffaloes. The incidence of retention of afterbirth was high (58.82 per cent); followed by post-partum metritis (25.22 per cent); Cervico-vaginal prolapse (7.56 percent); Prolapse of vagina (5.88 percent) and eversion of uterus (2.52 per cent,. Intra-uterine therapy with broad spectrum antibiotic preparations (Mastalone-U; Steclin and Terramycin) was found to be superior followed by recovery in 8-10 days and a very high post-recovery conception rate in these cases. The age old practice of manual removal of retained placenta be eliminated and replaced by intra-uterine antibiotic treatment alone for such cases in neo-veterinary gynaec therapy.

* * *

Buffaloes play a very vital role in dairy economy. Buffalo is still a formidable competitor for the Cross-bred cow due to milk richer in fat content and its organoleptic preference of consumers.

One of the major factors affecting the milk yield in recently calved buffaloes is the post-parturient uterine disorders. Sudden post-partum reduction in milk yield is the cardinal indicator suggestive of the onset of insidious low grade endo-metritis. The present studies were therefore undertaken to ascertain the types of post-partum uterine disorders, their incidence and effective therapy for recovery.

Materials and Methods

The material comprised of gynaeco-clinical cases of post-parturient buffaloes presented at University Gynaecologic Clinic, local dairies and Govt. Veterinary Poly. Clinic, Akola. 119 cases extending over a two-year period (1972-74) were studied. Each buffalo was subjected to detailed gynaeco-clinical examination on the diagnosis of which the cases were classified into groups of Retention of afterbirth, Post-partum metritis, Prolapse of vagina, Eversion of uterus and Cervico-vaginal prolapse. The cases were divided into groups of ten buffaloes each except the last group which had nine cases. Varying chemo-therapy was tried sequentially group-wise as the cases were presented in the clinics, to ensure a fairly even frequency distribution of the cases per treatment therapy. Cases of prolapse and eversion were applied rope trusses following treatment to help in the intra-pelvic retention of genitalia and avoid possible recurrence.

Intra-uterine therapy was carried out using one of the standard antibiotic proprietory preparations group-wise. The therapy is detailed category-wise under Results.

Gynaeco-clinical examination of each case was carried out daily for four days

during and after treatment and at periodic intervals of 3 to 5 days thereafter till a clinical cure was effected. The diagnosis and also the stage of clinical cure were based on clinical findings and palpable characteristics of tubular genitalia and gonads prior to and following treatment. Patho-bacteriological aspects therefore did not fall in the perview of these studies. The time taken for clinical cure was recorded in days. Fertility results were confirmed by Pregnancy Diagnosis examination conducted two months after natural service or A.I. following the clinical cure of the case.

The utero-vaginal status was categorised into Tonic, Atonic and Flabbly (Kaikini, 1972). Cases of retetntion of afterbirth were classified on the basis of placental status into: Normal, Foetid Necrotic.

The data was subjected to standard statistical analysis as per Snedecor and Cochran (1967).

Results and Discussion

Of the 119 buffaloes studied, the break-up and incidence of post-partum reproductine disorders was: Retention of afterbirth 70 cases (58.82 per cent); Post-partum metritis 30 cases (25.22 per cent); Prolapse of vagina 7 cases (5.88 per cent); Eversion of uterus 3 cases (2.52 per cent and Cervico-vaginal prolapse 9 cases (7.56 per cent).

1) *Retention of Afterbirth*

Of the 70 cases of retention of afterbirth studied in 39 (55.50 per cent) cases, the placenta was normal; in 9 (13.00 per cent) cases it was foetid and in 22 cases (31.50 per cent) it was necrotic.

(i) *Compron Therapy:* 30 cases in three groups of 10 each, were treated each with intrauterine therapy of two Compron (M. & B) pessaries inserted daily for three to four days, depending on the response of each case. The mean value for recovery was 8.6 days with conception rate of 90 percent.

(ii) *Steclin Therapy:* 30 cases in three groups of ten each were treated each with intrauterine therapy of two Steclin (Squibb) boluses inserted daily for three to four days depending on the response of each case. The mean value for recovery was 7.70 days followed by conception in each (100 per cent).

(iii) *Terramycin (Pessary) Therapy:* 10 cases (one group) were treated each with intrauterine therapy of two Terramycinn (Pfizer) pessaries inserted daily for three to four days depending on the response of the case. The mean value for recovery was 8.10 days and all the ten cases conceived thereafter.

Broad spectrum antibiotic intrauterine therapy was found to be superior in retention of placenta cases without manual removal.

2. *Post-partum Metritis*

Of the 30 cases of post-partum metritis, 20 were treated with Mastalone U and 10 with Terramycin liquid (Pfizer) 10 ml. each dissolved in 20 ml. distilled water and given intra-uterine daily for three to four days depending on the response of the case. Mean value for recovery was 8.25 days and 8.90 days respectively with all the recovered buffaloes conceiving to service or A.I. thereafter.

Findings of the present studies are in full agreement with that of Schlottauer (1926), Madhavrao (1945), John (1953). Lautebach (1961), Banerjee (1963), Hammerman (1963), Mulling and Benthien (1964) and Kaikini (1967).

3. *Prolapse of Vagina*

Seven cases (5.88 per cent) of vaginal prolapse were treated with Terramycin after reducing the prolapse. Two Terramycin pessaries (Pfizer) were inserted in the cervix for a period of three to four days depending on the response of each case. The mean value for recovery was 4.86 days, subsequently followed by conception.

4. *Eversion of Uterus*

Three cases (2.52 per cent) of eversion of uterus were treated with two Steclin boluses inserted intra-uterine and Dicrysticin (Squibb) 20 lac I.U. injected intra-muscular daily for a period of three days after reducing the eversion. Mean value for recovery was 8.30 days. These buffaloes conceived thereafter.

5. *Cervico-Vaginal prolapse*

Nine cases (7.55 oer cent) of cervico-vaginal prolapse were treated with two Terramycin pessaries intra-uterine and intramuscular injection of Combiotic-V 20 lac. I.U. (Pfizer) daily for a period of three days after reducing the prolapse. Mean value for recovery was 8.88 days. Post-recovery conception of the cases was normal and uneventful.

These findings are in partial agreement with those of Narasimhan (1965) and Narasimhan *et al* (1967) but differ from that of Sane (1967).

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REFERENCES

Banerjee, A.K. (1963). A study of the action of Terramycin on the bacterial flora of the uterus in cattle following retained placenta Thesis. Utrecht. pp. 118 Vet. Bull. 34: 3520.

Hammerman, J. (1963): Treatment of retained placenta with special reference to the fertility of the cow. Proc. 9th Nordic Vet. Congress Copenhagan Vol. II: 434-436. Vet. Bull. 33: 4552.

John, K.B. (1953): Retained placenta and aversion of uterus in the dairy cow-Cornell Vet. 44: 125-128.

Kaikini, A.S. (1967) Notes on Veterinary Gynaecology, Bovine Infertility, Obstetries and A.I. Pub. Students Coop. Stores, Nagpur Vet. College, Nagpur-440006.

Kaikini, A.S. (1972) Unpublished data.

Lautebach, (1961) Introduction into the uterus of various antibiotic combinations after difficult birth vet. Bull. 32: 954.

Madhava-rao (1945) Retained placenta — Use of sulphanilamide in cases of — Indian Vet. J. 22: 202.

Mulling, M and Benthien, H.A. (1964): Uese of uterine ureomycin tabletsin cattle. Vet. Bull. 34: 3466.

Narasimhan, K.S. (1965): A method of reposition of prolapsus uteri in the Indian Water Buffaloes. Indian Vet. J. 42: 214-218.

Narasimhan, K.S., Thangaraj, T.M. and Subramanyam, R. (1967) A Method of Retention of Recurrent Prolapse of the uterus in Bovines. Indian Vet. J. 44: 67-73.

Sane, C.R. (1967). Problems of Reproductive failuresin Cattle and Buffaloes. Proc. 12th ICAR. Conference on Animal Diseases, Poona.

Schlottauer, C.F. (1926): A methodof handling retained foetal membranes in the cow. Indian Vet. J. 3: 146-149.

Snedecor, G.W. and Cochran, W.G. (1967): Statistical Methods 6th Edn. Pub. Oxford and IBM Co. Bombay.

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Prostaglandin Administration On Oestrus Induction And Fertility In Suboestrus Cows

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ABSTRACT

Fifty four cross bred cows beyond 70 days postpartum which had active corpus luteum of 7-14 days of age were given 500 microgram of 'Estrumate', a prostaglandin F_2 alpha analogue and results were compared with the untreated cows of the herd. In all, 98.5 percent cows exhibited oestrus at an average intrval of 53.2075 ± 1.038 hours after the administration of the drug. Among these 92.45 percent ovulated at an average interval of 82.6122 ± 2.0015 hours after drug administration. The mean post partum oestrous interval of the experimental animals was 76.0283 ± 1.9296 days compared to 88.303 ± 3.9818 days among the untreated animals of the herd. 43.4 percent of treated animals conceived at the induced heat. The number of inseminations required for conception of the experimental animals did not vary significantly from the rest of the herd. The service period of the treated animals was significantly shorter ($92.4583 + 3.5394$ days) than rest of the herd (135.11 ± 6.9742 days). Thus it could be inferred that 'Estrumate' was effective in the induction of oestrum with satisfactory fertility.

* * *

Prostaglandin F_2 alpha is thought to be the natural luteolysin in Cattle and a number of synthetic analogues have been found to be potent luteolytic agents. Based on this, several investigations have been made into the use of prostaglandin F_2 alpha or its analogues for treatment of suboestrum in cattle. (Cooper and Furr (1974); Kruif and Brand, 1976; Eddy, 1977). The present investigation was undertaken to find out the efficacy of 'Estrumate' a prostaglandin F_2 alpha analogue in the treatment of suboestrum in cross bred cows.

Materials and Methods

Fifty four cross bred cows beyond 70 days postpartum belonging to the University Farm, and having Corpus luteum of 7-14 days of age as revealed by repeated rectal examination were administered a single dose each of 500 microgram of *Estrumate intra muscularly. The treated cows were closely observed for signs of heat. Intensity of oestrum, duration of Oestrum and time of ovulation were observed. All the cows in heat were inseminated with good quality chilled semen and pregnancy rate was observed. Those failed to settle to first insemination were reinseminated on subsequent heat and pregnancy diagnosed. The service period and the number of inseminations

* Cloprostenol, 500 microgram in 2 ml. ICI pharma Luzern-6002.

needed for conception in experimental animals were compared with rest of the herd.

Results

Out of 54 suboestrous cows treated, 53 (98.15%) showed visible signs of heat within a mean period of 53.2075±1.038 hours (range 48-72 hours) after the administration of the drug. Among this, 43.40 percent showed pronounced heat, 32.68 percent medium and 24.53 percent weak signs of heat. The average duration of oestrum was 17.8133±0.2964 hours. Forty nine cows (92.54%) ovulated and the interval from the administration of the drug to ovulation was 76 to 92 hours with a mean of 82.6122±2.0015 hours. Twenty three (43.40 percent) cows conceived at the induced oestrus while another 22 cows conceived at the subsequent insemination giving an overall conception rate of 80.33 percent. The post partum oestrous interval was considerably shorter (76.0283±1.9296 days) in the experimental animals than that of rest of the herd (88.303±3.9818 days). The number of inseminations required for conception (1.56±0.1453) in the treated animal did not significantly vary from the rest of the herd (2.4156± 0.0867). The service period of the experimental animals was significantly shorter (92.458±3·5394 days) than the herd average (135.11±6.9742 days).

Discussion

In the present investigation 500 microgram of Estrumate was used for induction of oestrus which is based on the earlier reports of Peters *et al.* (1977); Barnabe *et al.* (1978) and Seguin and Gustaffason, (1978). The dose was found to be very effective in the induction of oestrum since 98.15% of treated cows evinced oestrum. It was further observed that on an average 53.2075±1.638 hours were required to induce oestrus after the administration of the drug which agrees with earlier reports of Cooper and Furr (1974); Leaver *et al.* (1975) and Gupta *et al.* (1978). The poor oestrous response to PGF_2 alpha treatment reported by earlier workers (Eddy, 1977; Leidl *et al.* 1978; Khurana, 1979) might due to selection of cows in the non responsive stage of oestrous cycle. It is quite evident from the study that only 23 cows exhibited pronounced oestrum while 30 cows showed medium to weak signs of heat. The failure of expression of pronounced heat in a large population of experimental animals might be due to subclinical infection of uterus resulting in partial luteolysis. (Ginther, 1968). The duration of oestrum of the induced heat (17.8133± 0.2964 hours) agrees with the average value reported for cows of similar genetic groups (Mathai & Raja, 1978; Iyer and Madhavan, 1981). Thus it could be assumed that the duration of induced oestrum did not show marked variation from natural oestrus in cross bred cows. In all, 92.54 percent cows ovulated within a period of 82.6122+2.0015 hours. Lauderdale *et al.* (1974); Peters *et al.* (1977); Kaneda *et al.* (1978) and Nakama *et al.* (1978) also obtained similar results. The conception rate at the induced oestrus (43.40 percent) observed in the present study are consistent with Grunert *et al.* (1978) and Kupfer Schimied *et al.* (1979). The mean cycle length of those which failed to conceive for the first heat was 17.46 days and the overall conception rate was 83.33 percent. As reported by Esslemont *et al.* (1977), the post partum oestrous interval could be considerably reduced (76.0283±1.92 96 days) by treatment with Estrumate compared to that of the herd (88.303±3.9818 days).

The number of inseminations required for conception in the treated cows (1.56+ 0.1433) is comparable to that reported by Anderson (1979) and also did not differ significantly from the rest of the herd. This indicates that fertility in terms of number of insemination per conception is not adversely affected by chemical induction of oestrum. Similar observations were also made by Inskeep (1973) and Roche (1974). The significant reduction of service period of the experimental animals compared to that of the herd is akin to that of Esslemont *et al.* (1977). Thus it could be inferred that administration of Estrumate to suboestrous cows in the early post partum period would be beneficial in improving the breeding efficiency of the herd.

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REFERENCES

Anderson, J. (1979). Oestrus Synchronisation in cattle. *Vet. Bull.* **50** abst. 5167.

Barnabe, R.C.; Barnabe, V.H. and Mucciolo, R.G. (1978). Use of PGF_2 alpha for Oestrus synchronisation. Effects according to the site of injection in the genitalia. *Amim. Breed* Abstr. **46** abst. 2671.

Cooper, M.J. and Furr, B.J.A. (1974). The role of postaglandins in animal breeding *Vet. ERec.* **94** 161.

Eddy, R.C. (1977) Cloprostenol as a treatment for no visible oestrum and cystic ovarian disease in dairy cows. *Vet. Rec.* **100:** 62-65.

Esslemont, R.J. Eddy, R.G. and Ellis, P.R. (1977) Planned Breeding in autum calving dairy herds. *Vet. Rec.* **100** 426-427.

Ginther, O.J. (1968). Utero ovarian relationship in cattle. Physiological and applied Veterinary aspects. *J. Am. Vet. med. Ass.* **153:** 1656.

Grunert, E., Diee, G., Fietbrock, W. and Dohonest, H. (1978). Estrumate treatment of Cattle with silent heat. Zuchthyg, **13:** 49-54.

Gupta, K.P., Farroqui, S.U. and Pant, H.C. (1978) Synchronisation of Oestrum in cattle and buffaloes with ICI 80996 an analogue of PGF_2 alpha. *Vet. Res. Bull.* **1:** 103-105.

Inskeep, E.K. (1973). Potential use of Prostaglandins in control of reproductive cycle of domestic animals. *J. Anim. Sci.* **36:** 1149-1150.

Iyer, C.P.N. and Madhavan, E. (1981). Reproductive performance of Jersey × Sindhi cross bred cows *Milcow* **3:** 327-330.

Kaneda, Y.; Domeki, I.; Kamomae, H. and Nakahara, T. (1978). Synchronisation of Oestrus and ovulation with intra muscular injection of Prostaglandin F_2 alpha followed by additional synthetic LH releasing hormone in the cow. *Jap. J. Anim.* Reprod. **24:** 121-128.

Khurana, N.K. (1979). Studies on the role of PGF_2 alpha and gonadotropin releasing hormone for induction of oestrus and ovulation: *MVSc. Thesis Hariyana Agrl. Univ. Hissar.*

Kruif, A. De and Brand, A. (1976). Treatment of Suboestrum in Cattle with prostaglandins Anim. Breed Sbst. **44:** abst. 4749.

Kupfer Schmied, H. Bachmann, P.H. and Muther, E. (1979). Investigations concerning heat induction and synchronisation of cattle. *Schweizer Arch. Tier heulk.* **121:** 239-242.

Lauderale, J.W., Seguin, B.E.; Stellflung, J.N.; Chenault, J.R., Thatcher, W.W.; Vincent, C.K. and Loyancano, A.P. (1974). Fertility in cattle following PGF_2 alpha injection. J. Anim. Sci. **38:** 964-967.

Leaver, T.D.; Glencros, R.G. and Pope, O.S. (1975). Fertility of Friesian heifers after luteolysis with a Prostaglandin alalogue (ICI 80996). *Vet. Rec.* **96:** 383-384.

Leidl, W. Bostedt, H., Stolla, R; Kutin, A. and Schefel, S.W. (1978). The effect of PGF_2 alpha and clincial experience with analogue Estrumate specially in anoestrum in cows *Amin. Breed. Abstr.* **47:** 3601.

Mathai, E. and Raja, C.K.S.V. (1978). Pattern of oestrous cycle in Jersey × Sindhi heifers. Kerala J. Vet. Sci. **9:** 159-160.

Nakama, S., Imori, T.; Nilia, S., Yanagia, G., Inkaba, T., and Uchida, A. (1978). Experimental induction of oestrus and ovulation in cattle by systemic in jection of PGF_2 alpha and its analogue. Jap. J. Reprod. 24 161-168.

Peters, J.B.; Welch, J.A.; Lauderdale, J.W. and Inskeep, E.K. (1977). Synchnonisation of oestrus in beef cattle with PGF_2 alpha and oestradiol benzoate J. Anim. Sci. **45:** 230-235.

Roche, J.F. (1974). Synchronisation of oestrum and fertility following Artificial insemination in heifers given PGF_2 alpha J. Reprod. Fert. **37:** 135-138.

Seguin, B.E. and Gustaffson (1978) Use of PGF_2 alpha analogue in dairy cattle with unobserved oestrus Theriogenology **10:** 54-65.

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Fertility Trials In Bovines

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ABSTRACT

Fertility trials were conducted at field and farm levels involving eight selected bulls-five Surti and three H.F. Required numbers of semen ejaculates were collected, evaluated and frozen on LN2 vapour in 0.5 ml. capacity medium straws, using Tris Fructose Yolk Glycerol (TFYG) dilutor. Totally 342 buffaloes and buffalo heifers were inseminated using 426 frozen semen doses. Overall fertility percentage achieved was 57.24%, requiring 2.69 inseminations per conception on an average. The fertility amongst the buffalo bulls under study was 62.02%, 52.17%, 61.43%, 53.33% and 50.00% respectively for five Surti buffalo bulls viz-SB_{AS}, SB_{AJ}, SB_{RM}, SB_{PR}, and SB_{AT}. Number of inseminations required per conception were 2.57, 2.83, 2.27, 3.37 and 3.20 for buffalo bulls respectively. In the villages of two advanced districts of Gujarat State 285 doses of frozen H.F. bull semen were utilized to inseminate 251 kankrej cows and heifers. On an average 2.46 inseminations were required per conception. The over all fertility achieved was 46.21% for the three bulls, HF 316, HF 317 a d HF 307, the fertility percentages were 52.87, 48-75, and 36.90 The number of inseminations required per conception were 2.06 2.93 and 2.50 for the three bulls respectively. On a farm 98 insemination doses of frozen H.F. bull semen were required to settle 28 repeating Kankrej cows and heifers. Nine repeat breeders could not be conceived. On an average 3.5 inseminations were required per conception. The fertility percentages between eight bulls under study, varied apparently.

* * *

Obviously, the most reliable and a practical measure of semen quality is to assess fertility by examining for pregnancy in the inseminated females. Not many scientific reports are available on large scale field fertility trials, particularly in buffaloes and cows using frozen semen.

Materials and Methods

Fertility trials were conducted at field and farm levels in normally cycling bovines and repeat breeder cows using frozen semen from the selected bulls. Out of 23 bulls under study; eight bulls were selected for fertility trials. They were SB_{AS}, SB_{Aj}, SB_{RM}, SB_{PR}, and SB_{AT} of Surti breed and HF 316, HF 317 and HF 307, of Holstein Friesiau breed. The required number of semen ejaculates were obtained in twice a week collection schedule, in sterilized collection tubes using artificial vagina. The semen was evaluated as per the standard techniques for volume, colour, consistency, mass activity (Herman & Maddeu-1953), Live and abnormal spermatozoal percentages. (Hancock—1951) and individual progressive motility of spermatozoa. Semen

TABLE 1 Fertility of frozen semen for different bulls

Sr. No.	Bulls	No. of Animals inseminated	No. of Animals followed	No. of Animals Preg.	Fertility %
1.	SBAt	99	79	49	62.02
2.	SBAj	85	69	36	52.17
3.	SBRM	78	70	43	61.43
4.	SBZR	45	30	16	53.33
5.	SBAT	35	28	14	50.00
	Total	342	276	158	
	Overall mean Fertility				57.24
6.	HF316	87	87	46	52.87
7.	HF317	80	80	39	48.75
8.	HF307	84	84	31	36.90
	Total	251	251	116	
	Overall mean fertility				46.21%

TABLE 2 Number of inseminations required per conception for different bulls.

Sr. No.	Bulls	No. of insemina-tions	No. of animals conceived	No. of insem/ concep.
1.	SBAs	126	49	2.57
2.	SBAj	102	36	2.83
3.	SBRM	98	43	2.27
4.	SBPR	54	16	3.37
5.	SBAT	46	14	3.20
	Total	426	158	
	Av.			2.69
6.	HF316	96	46	2.06
7.	HF317	98	39	2.93
8.	HF307	91	31	2.50
	Total	285	116	
	Av.			2.45

samples with optimum quality (Minimum +++Mass activity and 70% Motility) were processed further, diluting them in Tris Fructose yolk glycerol (TFYG) dilufor (FAO-1979), keeping about 50 to 60 million spermatozoa per ml. of semen before freezing. The diluted semen was filled in 0.5 ml. medium straws and sealed dipping them in polyvinyl alcohol (PVA) powder. The equilibration period provided was 5 hrs. at 5°C. A "Thermocol freezing unit" using LN2 vapour freezing method (Shetti et al-1981) was adopted for freezing of the semen. The Frozen semen was stored at least for 10 days before it was used for inseminations. Thawing of frozen semen was effected by immersing the straws in water at 102°—104° F. in a thermos for 15 to 20 seconds.

The frozen buffalo bull semen was utilized for inseminating Surti buffaloes and heifers at the college A. I. Centre, where Veterinarians were involved in insemination and pregnancy diagnosis work. Totally 342 buffalo and buffalo heifers were inseminated using 426 frozen semen doses. The frozen Holstein bulls' semen doses were distributed for inseminations in Kankrej cows and heifers at field level in the selected villages of the two advanced districts of Gujarat state. The trained inseminators at village A. I. Centres were involved in the insemination and pregnancy diagnosis work. Totally 251 cows and heifers were inseminated using 285 frozen semen doses. About 98 frozen semen doses of two Holstein bulls were utilized to study the efficacy of Frozen semen to settle the repeating Kankrej cows and heifers of Livestock Research Station, Gujarat Agricultural University, Anand. The animals had repeated for 7 to 18 times. A farm veterinarian performed the inseminations and pregnancy diagnosis work. The data were analyzed as per the standard method described by Snedecor and Cochran (1971).

Results and Discussion

In buffaloes and heifers, the overall fertility percentage achieved was 57.24%,

requiring on an overage 2.69 inseminations per conception. The buffalo bulls under study varied for their fertility. For five buffalo bulls—viz. SB_{AS}, SB_{AJ}, SB_{RM}, SB_{PR}, and SB_{AT}, the fertility percentages were 62.02%, 52.17%, 61.43%, 53.33% and 50.00% (Table: 1) and the number of inseminations required per conception were 2.57, 2.83, 2.27, 3 37 and 3.20 respectively (Table—2). The overall fertility achieved in the present experiment is comparatively higher than fertility rates obtained by Takkar et al (1980) as 36.12%, Vasanth (1979) as 45%, Chinnaiya et al (1979) as 46.22% Patil et al (1981) as 55.55% and Reddy et al (1982) as 47% in buffaloes using Tris diluted frozen semen. The overall fertility percentages obtained by Heuer (1982) as 53.8% 54.3%, 56.7% and 53.80% for buffalo semen diluted and frozen in LFYG; Tris; Tris milk and skim milk diluents respectively are well in accordance with the results of present experiment. Ahmad et al (1982) reported in buffaloes, the average requirement of numbers of inseminations per conception to be 1.77 to 2.75, which is very well in agreement with the findings of the present study.

The overall fertility achieved following 285 inseminations performed in 251 Kankrej cows and heifers was 46.21%. On an average 2.46 insemintions were required per conception. For three bulls, HF—316, HF 317 H.F. 307, the fertility percentages were 52.87, 48.75 and 36.90 respectively. The number of inseminations required per conception were 2.06, 2.93 and 2.50 for the three bulls, respectively. The centrewise fertility results are presented in Table 3.

These fertility results obtained are well comparable with those reported by Guha (1972) as 46.00% Chinnaiya et al (1974) as 30 to 60% and Maulick et al (1975) as 42.2%, who used frozen semen from Friesian sires in indigenous cows. However, the present fertility results in cows are quite low as compared to the results reported by Ishii et al (1979) as 52.58%

TABLE 3 Fertility results for frozen semen

Bulls	No. Insem.	No. Preg.	Ferti. %
	Centre A		
HF 316	14	5	35.71
HF 317	31	19	61.29
HF 307	28	14	50.00
Total	73	38	52.08
	Centre B		
	No. Insem.	No. Preg.	Ferti. %-
	20	9	45.00
	8	4	50.00
	6	4	60.66
	34	17	50.00
	Centre C		
	No. Insem.	No. Preg.	Ferti. %
	24	19	79.16
	32	11	34.36
	42	8	19.00
	98	383	8.77
	Centre D		
	No. Insem.	No. Preg.	Ferti. %
	29	13	44.83
	9	5	55.55
	8	5	62.50
	46	23	50.00
	Tottal		
	No. Insem.	No. Preg.	Feri. %
	87	46	52.87
	80	39	48.75
	84	31	36.90
	251	116	46.21

and Tomar (1981) as 61.34%.

The average number of inseminations per conception required in present study was 2.45 which is well comparable with the observations of Nair (1975), who reported that the Brown Swiss frozen semen required 2.49 inseminations per conception. However the fertility trials undertaken by Qureshi (1979) and Tomar (1981) showed the requirement of 2.76 and 3 to 4 insemination respectively, are higher than the present findings.

TABLE 4 Fertility in repeat breeding kankrej cows and heifers.

Bulls	No. of insemina-tions	No. of animals conceived cows	heifers	No. of insem/ concep.
HF 316	67	16	4	3.35
HF 317	31	6	2	3.87
Total	98	22	6	
Av.				3.50

In the present study, the use of frozen semen in repeating Kankrej cows and heifers required on an average 3.5 inseminations per conception (Table: 4), which is quite lower to settle a repeat breeder. These findings are much in contrast to the study of Bhosrekar (1973) who observed more cases of repeat breeders due to the inseminations with using frozen semen from Brown Swiss sires.

It was possible to settle 28 repeat breeding Kankrej cows and heifers out of 37, using frozen semen. The semen of H.F. 316 and H.F. 317 bulls required 3.35 and 3.87 inseminations per conception (Table: 2) However, the exact role of frozen semen in settling the repeat breeders in the present study is not very clear.

It was observed from the present fertility trials that there were significant differences in fertility rates amongst the bulls, studied for the purpose. This may be due to the bull difference in post thaw motility of the semen (Singh et al 1980, Reddy et al 1982) or the efficacy of an inseminator (King-1973). The lo er fertility results in some cases as reported, may be attributed to the various causes as suggested by Kumaran (1965), Bhosrekar (1973) and Nair (1975), which include lower Post-thaw motility of semen, faulty insemination technique, delayed inseminations after thawing and poor nutritional status and poor fertility of cows etc; the later being most important factors affecting the breed improvement programmes in our country.

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REFERENCES

Ahmad, N. Ahmad, R.C. and Tahir, M. (1982). Study on some non-genetic factors affecting the fertility of buffaloes. Paper presented in seminar on buffalo Reprod. and meat prod. Tanuku (A.P.) 15th to 17th Jan. '82.

Bhosrekar, M. (1973) Investigation into the incidence and causes of repeat breeding in dairy cattle at NDRI Karnal. Indian Vet. J. **50**: 418-429.

Chinnaiya, G.P., Sharma, P.A. and Ganguli, N.C. (1979). Fertility studies on frozen buffalo semen. Indian J. Dairy Sci. 32.

Chinnaiya, G.P., Sattar, S.A., Venkatraman, T.G. and Sampath, S.R. (1974). Studies on freezing of bovine semen and its use. Indian vet. J. 51 (7-8): 501.

FAO Animal Production and Health paper-13 (1979) pp. 313. FAO Rome.

Guha, H. (1972). Annual technical report of the UNDP project at Haringhatta for 1972, Haringhatta, West Bengal. central livestock Research cum Breeding station pp. 18.

Hancock, J.L. (1951). The morphology of the bull spermatozoa. J. Exp. Biol. **29**: 445.

Herman, N.A. and Madden, F.W. (1953). Artificial Insemination of dairy cattle. A handbook and Laboratory manual Lucas brothers, Columbia, M.O.

Heuer, C. (1982). Effects of extenders, glycerol and equilibration time on the quality of frozen water buffalo semen as measured by sephadex filtration, motility estimates and conception rates. Paper presented in "buffalo seminar on Reprod. and meat prod., held at Tamku (A.P.)

Ishii, K., Vawata, S. and Morita, Z. (1979). Studies on the use of bovine frozen semen 2. The fertilizing capacity of semen frozen by two methods and the effect of activated vit. B. Anim. Breed. Abstr. **47**: 3590.

King. G.J. (1973) "The most important link in the froeen semen chain" Workshop. Reprod. Physiol. Dairy animals at Karnal (C.F. Qureshi, 1979).

Kumaran, S. (1965). A comparative study of the fresh and frozen bovine semen morphology and fertility. Indian Vet. J. **42**: 731.

Maulick, S.K., Deb. N.O., Sanyal, B. ad Sen A.K. (1975). Conception rate of zebu and cross-bred cattle with imported froeen semen. Indian J. Anim Sci. **45**: 422-426.

Nair, B.R.K. (1975). A study on the conception rate in cattle due to insemination with deep frozen semen. Indian vet. J. **52**: 165.

Patil, D.S., Hukeri, V.B. and Deshpande, B.R. (1981). A suitable dilutor for deep freezing of buffalo semen. Indian J. Anim. Reprod. **1**: 107.

Qureshi, S.H. (1979)..A study on the conception rate in kumaon Hill cattle, inseminated by deep frozen semen of Brown Swiss bulls. Indian vet. J. **56**: 37-40.

Reddy, D.N.C., Ramanatha Gupta, D.C.L. and Radhakrishna, D.T.G. (1982). Deep freezing of buffalo semen and its fertility studies. Indian vet. J. **59** (7): 574-575.

Shetti, A.B., Hukeri, V.B. and Deshpan de, B.R. (1981). Deep freezing of buffalo spermatozoa in "Triladyl" diluent. Ind. J. Dairy Sci. **34** (1): 108.

Singh, H.S., Fluekiger, A. and Wieser, M.F. (1980). Deep freezing of buffalo bull semen using four different dilnents. Indian vet. J. **57** (2): 143.

Snedecor, G.W. and Cochran, W.G. (1971). Statistical methods. 6th edn. The Iowa state Univ. Press, AMES, IOWA, U.S.A.

Takkar, O.P., Matharao, J.S., Chauhan. F.S., Tiwana, M.S. and Meharsing (1980). Reproductive performance in buffaloes. Paper read in IInd All India Sympo. Anim. Reprod. held at Bangalore.

Tomar, S.S. (1981) Fertility results of deep frozen semen of Holstein Friesian bulls. Indian J. Anim. Res. **15**: 50.

Vasanth, J.K. (1979) Note on freezing of buffalo semen and fertility. Buffalo Reprod. artif. insem. FAO Animal Prod. Htlh. Paper-13. pp. 304-309.

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The Studies On Plasma Levels of ACTH And Corticosteroid In Early Neonatal Buffalo-Calves.

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ABSTRACT

Following the standard Radio-immuno assay technique, the blood plasma levels of Adreno-Corticotrophic hormone (ACTH) were estimated in early neonatal buffalo calves of zero to fifteen days of age. Simultaneously, the levels of plasma corticosterone were also determined by using fluorometric method. The levels of ACTH and corticosterone were observed to be at peak on the day of birth and continue to remain higher upto first two days of life ($P<0.01$). Thereafter, the levels of these hormones declined abruptly in the beginning and gradually lateron between third to fifteen day of neonatal life. The interrelationships between the levels of these hormones are discussed. It is evident from these findings that the buffalo calves call for the active secretion of these hormones so that the stressful challenge of abrupt separation from the maternal environment can be met with successfully.

* * *

The influence of foetal hypothalamo-hypophyseal-adrenocortical axis on the initiation of parturition in farm animals has been fully established. (Liggins, et al. 1973). The new born animal is not only facing metabolic stress at the time of parturition but is also threatened with cold and hostile environment immediately after birth, (Mehta and Varman, 1982, 1983). The response of hypothalamo hypophyseal-adrenocortical axis during starvation, exposure to cold and transportation has been studied in new born cow calves, (Hartmann and Weidner, 1973). The incidence of calf mortality due to calf diarrhoea has been correlated with the high levels of corticosteroids, (Hudson, et al. 1976). The experimental adrenalactomy in early neonatal calves produce immediate detrimental effects on the health of these animals, (Estergreen and VanDemark, 1961). The purpose of this study was to investigate the response of early neonatal buffalo-calves to extra-uterine environment through the blood plasma levels of ACTH and corticosterone hormone as a part of neonatal adaptation.

Materials and Methods

To study the blood plasma levels of Adreno-corticotrophic hormone and Corticosterone, the blood samples were drawn from jugular vein of healthy buffalo calves from birth to fifteen days of age. The aliquots of blood plasma samples were stored in deep freeze until analysed further.

The plasma levels of ACTH were analysed through the use of ACTH Radio-immuno assay kits supplied by M/S. Radio Chemical Centre, Amersham, England. The human ACTH antigen was tagged with ^{125}I. The standard curve

and the analysis of ACTH were made according to the instructions supplied with the kit. The radio activity of these samples was determined through the use of Medical Spectrometer, (Electronic Corporation of India).

The plasma levels of corticosterone were estimated through the fluorometric quontitation following the standard technique, (Greaf and Stundiger, 1970). The mean and standard deviation of ACTH and Corticosterone concentration were calculated following standard stastistical methods, (Steel and Torrie, 1960).

Results and Discussion

The mean plasma levels of ACTH and corticosterone hormones alongwith their standard deviations observed for the buffalo-calves from birth to fifteen days of life are shown in Table-1.

The plasma ACTH and corticosterone levels were observed to be highest on the day of birth. Eventhough, the levels started falling thereafter, they were significantly higher ($P<0.05$) on the first and second day of life. A pecipitous fall in levels of both hormones was observed on third day of neonatal age to eight days of early life, but subsequently the plasma levels had shown a slight rise. The correlation between the plasma levels of ACTH and corticosterone hormone was significant ($P<0.01$) and positive, ($r=+0.704$). However, the correlation between age of these calves and the plasma levels of these hormones was negative, ($r=-0.720$).

TABLE 1 The mean blood plasma levels of Adrenocorticotrophic hormone (ACTH) and Corticosterone on the neonatal buffalo calves from birth to fifteen days of age.

Age in days	Plasma ACTH (Pg/ml) Mean ±S.D.	Plasma Corticosterone µg/100 ml Mean ±S.D.
Birth	110.62±2.82 (4)*	3.36±0.17 (4)
1	103.21±2.52 (4)	2.42±0.23 (4)
2	91.93±0.90 (4)	1.81±0.05 (4)
3	38.15±1.06 (6)	1.90±0.20 (4)
4	n.d.	1.13±0.08 (4)
5	n.d.	0.85±0.10 (4)
6	11.60±0.48 (4)	1.15±0.30 (3)
7	16.50±1.80 (4)	n.d.
8	20.92±1.62 (5)	1.02±0.80 (4)
11	24.94±1.91 (6)	1.25±0.06 (4)
13	21.19±0.08 (4)	1.45±0.05 (4)
15	18.46±1.62 (8)	1.36±0.21 (3)

n.d. Not Determined.

* Figures in the parenthesis indicate the number of observations.

The present studies on plasma levels of ACTH and corticosterone hormones in the early neonatal buffalo calves have revealed that a close associationship between these two hormones secretions is established right from birth. The peak levels of these two hormones on the day of birth and a day after indicate that the calves have responded to parturition and extra-uterine stressors. The high levels of these hormones at birth also seem to indicate that they influence the onset of parturition in buffalo species as observed in sheep and cattle, (Liggins, et al. 1973). The peak levels of these hormones also seem to protect the newborn animals against infections and respiratory distress, (Villee, 1976). A drop in the levels of these hormones between three to eight days of age indicate that the animals have adapted to environmentaland nutritional stressors. The blood plasma levels of corticosterone remain elevated far longer in the cow calves suffering from calf diarrhoea, (Hudson, et al. 1976). The extremely high levels of corticosterone in puppies adversely affect the absorption of immunoglobulins from the gut wall, (Gillette and Filkins, 1966). Such studies in cow calves and lambs

have not shown similar correlationships and the behaviour of buffalo-calves in this regards is not known, (Deutsch and Smith, 1957).

The present studies reveal the fact that the feedback relationship between ACTH and corticosterone are established right from birth in buffalo calves and such regulatory mechanisms must be developing in foetal life as observed with foetal lambs, (LLanos, et al. 1979).

REFERENCES

Deutsch. H.F. and Smith, V.R. (1957). Intestinal permiability of proteins in the new born herbivore. Am. J. Physiol. **191:** 275.

Estergreen, V.L. Jr, and VanDemark, N.L. (1961). Adrenalactomy of the calf and its effects on various blood constituents. J. Dairy Sc. **44:** 928.

Gillette, D.D. and Filkins, M. (1966). Factors affecting antibody transfer in new born pully. Am. J. Physiol. **210:** 419.

Greaf, V. and Studinger H.J. (1970). Z. Klin. Chem-4. Klin. Biochem, 8.368. In clinical Biochemistry, Principles and methods. H.ch. Curtius and Mare Roth (Eds.) 1974 p. 685. Water de Grutyler, Berlin. New York.

Hartmann, H. and Weidner. S. (1973). Effect of chronic stress on the calf with particular reference to hypothalamo-adenohypophysis system., Methematish Nutturwissen-Schafttiche, Reine. **22:** 515.

Hudson, S., Mullord, M. Writtestone, w.g. and Payne, E. (1976). Plasma corticosteroid level in healthy and diarrhoeic calves from birth to twenty days of age. Brit. Vet. J. **132:** 551.

Liggins, G.C. Fairclough, R.J. Grevies, S.A. Kendall, J.I. and Knox, B.S. (1973). The mechanism of Initiation of Parturition in the ewe. Rec. Prog. Horm. Res. **29:** 111.

Llanos. A.J., Ramchandran, J. Creasy, R.B. Rudolph, A.M. and M. Sernon-Ferre. (1979). Alpha Melanocyte Stimulating Hormone and Adrenocorticotrophin in regulation of Glucocorticoid secretion During Partinatal period in Sheep. Endocrinol. **105** (3): 613.

Mehta, V.M. and Varman, P.N. (1982). In Vitro glucose and Acetate metabolism in liver and perirenal adipose tissue of neonatal Buffalo Calves. J. Nucl. Agril. Biol. **10** (4): 139.

Mehta, V.M. and Varman, P.N. (1983). Thyroid Status of Early Neonatal Boffalo calves. Ind. J. Ani. Reprod. **4** (1): 50-52.

Steel, R.G.D. and Torrie, J.H. (1960). Principles and Procedures of Statistics. McGraw-Hill Co. N.Y.U.S.A.

Villee, D.B. (1976). Human Endocrinology, A Developmental Approach. W.B. Sounders Co. U.S.A.

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Peripheral Plasma, Sodium, Potassium, Calcium And Inorganic Phosphorus Profiles In Cows Retaining Placenta

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ABSTRACT

Of the four minerals (Sodium, Potassium, Calcium and in-organic phosphorus) studied, calcium and inorganic phosphorus levels could be used to predict placental retention in cows.

* * *

Etiology of placental retention in the bovine species remains elusive even today. The role of certain minerals etc. in placental retention is not clear. Experimental evidence in favour of perturbed plasma sodium, potassium, calcium and inorganic phosphorus has not been univocal (Boiter *et al.* 1972, Wilson *et al.* 1977 and Dutta 1980). It is intriguing whether plasma profile of one or more of these elements can be used to predict with precision impending placental retention in the bovine.

The present investigation was carried out with a view to determine the plasma levels of the minerals near parturition and to elevate their predictive value vis-a-vis placental retention.

Materials and Methods

Blood samples were collected 7-15 days prior to expected day of calving from a number of Brucella-free cows at the Punjab Agricultural University Dairy Farm. The cows which failed to expel their placentae within 12 hrs. of parturition (n=21) were grouped as having 'Retained Placenta'. Serum was separated by centrifugation at 2500 rpm and stored at $-20\,^{\circ}C$ pending analysis.

Serum samples collected on days -10, -5, -1, day zero (day of partus) and on days $+1$ and $+3$ of parturition were analysed for sodium and potassium levels by Flame Photometric method (Oser 1976). Calcium and inorganic phosphorus levels in the serum were determined as per Webster (1962) and Fiske and Subarow (1925), respectively. The data were:

Serum samples obtained from normally cleansing cows were also subjected to same analyses. The data were subjected to Students' 't' test.

Results

It follows from Table 1 that the average ($\pm$SD) sodium concentration 10 days before parturition in cows which retained foetal membranes (148.50 ± 6.75 mEq/lit) was not significantly different from that in cows which cleansed spontaneously (145.25 ± 5.08 mEq/lit). No significant drop in serum sodium levels occurred in either groups with approaching parturition. However, the mean sodium levels in both the groups touched minimum values on the day of parturition and this

TABLE 1 Mean ±SD periparturient sodium and potassium concentration in cows retaining and non-retaining foetal membranes.

Group	Item	Days around parturition					
		− 10	− 5	− 1	0	+1	+3
Retaining foetal membranes*	Sodium (mEq/L)	148.50 ±6.75	142.00 ±5.40	140.37 ±4.30	139.25 ±4.05	136.75 ±8.70	137.25 ±6.08
	Potassium (mEq/L)	4.60 ±0.65	4.55 ±0.08	4.48 ±0.38	4.20 ±0.21	4.25 ±0.22	4.38 ±0.23
Not retaining foetal membranes*	Sodium (mEq/L)	145.25 ±6.80	136.00 ±6.80	137.50 ±4.50	136.00 ±5.80	132.23 ±9.08	131.00 ±7.05
	Potassium (mEq/L)	4.35 ±0.25	4.25 ±0.10	4.15 ±0.09	4.00 ±0.09	4.08 ±0.12	4.28 ±0.15

* The differences between two groups of cows are non-significant (P 0.01)

TABLE 2. Mean ±SD periparturient calcium and phosphorus concentration (mg%) in cows retaining and notretaining foetal membranes.

Group	Item	Days around parturition								
		−10	− 5	− 4	− 3	− 2	− 1	0	+1	+3
Retaining foetal membranes	Calcium (mg%)	9.50* ±0.90	9.45 ±0.28	9.25 ±0.90	9.0 ±0.29	8.89* ±0.70	8.40* ±0.56	8.28* ±0.60	8.92** ±0.80	9.30* ±0.86
	Phosphorus (mg%)	5.38 ±1.00	5.00 ±0.80	5.10 ±1.20	4.90 ±0.75	4.95 ±0.90	4.80 ±0.78	3.25 ±1.00	4.50 ±1.30	5.00 ±1.20
Not retaining foetal membranes	Calcium (mg%)	10.50 ±0.73	9.82 ±0.78	9.50 ±0.94	9.40 ±0.92	9.40* ±0.98	9.30* ±0.93	9.20* ±0.50	9.38** ±0.65	10.12* ±0.28
	Phosphorus (mg%)	6.00 ±1.20	5.90 ±1.10	6.00 ±0.90	5.75 ±0.80	5.00 ±0.70	5.25 ±1.00	4.90 ±0.70	5.50 ±1.00	5.90 ±1,30

* (P>0.01) ** (P>0.05)

low concentration was maintained through days 3 postpartum. Despite somewhat higher serum sodium values in most of the cows with retained foetal membranes, the differences in the corresponding mean values with cows without placental retention were non-significant. The mean (±SD) potassium values in cows which retained placentae fluctuated between 4.20±0.21 and 4.60±0.65 mEq/lit during the periparturient period. These values, although marginally higher, were not different from the corresponding mean values in cows which expelled their foetal membranes normally. On no periparturient occasion did the mean values differ to any appreciable extent between the two groups of cows. However, the cows retaining foetal membranes maintained apparently constant values between day − 10 and day + 3, with marginally but non-significantly low values on day 0 and day + 1 of parturition. Majority of the cows (n=17) in the control group showed considerable day-to-day fluctuation in the potassium

concentration during the period of study; however, the minimum values were obtained on day 0 and day + 1 of parturition. On day + 3 of parturition the potassium values revealed a marginal rise in almost all the cows in both the groups.

The mean (±SD) calcium values in cows with and without retained placentae (Table 2) fluctuated between 8.28±0.60 mg per cent to 9.50±0.90 mg per cent and 9.20±0.50 to 10.50±0.73 mg per cent, respectively. The individual variations in serum calcium levels in both the groups of cows were negligible. With approaching parturition the calcium values tended to decrease. The lowest calcium values were observed on the day of parturition (day 0) in both the groups. The cows which subsequently retained placentae had generally lower mean calcium concentration on all the occasions before, during and after parturition. The differences between the corresponding mean values were significant on almost all the days of analyses.

With the advent of parturition a marginal decrease in mean serum inorganic phosphorus concentrations was evident in both the groups. While the mean ±SD inorganic phosphorus levels around parturition in cows without retained foetal membranes varied between 4.90± 0.70 mg per cent to 6.0±1.2 mg per cent, the cows with placental retention had the mean phosphorus values ranging from 3.25±1.0 to 5.38±1.00 mg per cent during the same period. The inorganic phosphorus values touched minimum values on the day of calving in both the groups of cows (Table 2). It is, however, evident from the data that cows which retained placentae had low prepartum mean phosphorus values on almost all the days of sampling and that the differences in the corresponding prepartum means between the cows with and without retention were statistically significant.

Discussion

The results of the present study indicate no major alterations in sodium or potassium values during immediate prepartum period in cows with or without placental retention. This is in consonance with the findings of Dutta (1980). There were no differences in the mean sodium or potassium values between cows retaining foetal membranes. This is in agreement with Dutta (1980) but disagrees with the findings of Boiter *et al.* (1972) who reported higher Na^+ and K^+ in cows with and without placental retention. Similarly, the results in buffaloes which indicate no differences in mean sodium or potassinm values, irrespective of whether placental retention occurred or not, are consistent with those of Dutta (1980). This does not however, mean that there is no relationship between these blood parameters and placental retention in the bovine, for a drastic shift in the immediate intercellular fluid may reduce the sensitivity of myometrium by interfering in someway with the reaction of oxytocin with its receptors. The involvement of K^+ and Ca^{2+} in the action of oxytocin on myometrium has been suggested (Berger and Marshall, 1961). Moreover, estrogen may increase influx of K^+ into cells without any substantial alteration in peripheral plasma values (Cole, 1950). This implies that the circulating Na^+ or K^+ values may not necessarily reflect their altered metabolism in the myometrium and as such may not be of any help in understanding their role in normal or abnormal parturition. The mechanism by which sodium and potassium concentration in blood is increased in cows with placental

retention (Boiter *et al.*, 1972) has not been elucidated. However, it is possible that due probably to extensive histamine production in Boider's study, Na^+ and K^+ retention in the kindey tubules or release of K^+ from blood or muscle calls might have lead to hypernaturaemia or hyperkalaemia in cows with placental retention.

The marginally higher Na^+ and K^+ concentration in cows with retained placentae, in the present study, may also be attributed to excessive histamine release from placental membranes even before parturition. Additionally, based on the reports that oxytocin under physiological conditions has a natriuretic effect (Michell and Noakes, 1980) it may well be the fact that the deficiency of oxytocin in placental retention (Boiter *et al.*, 1972) may result into Na^+/K^+ retention in the kidney, thus leading to a moderate hike in serum Na^+ and K^+ levels.

A decline in calcium concentration with the advancement of parturition in cows as evident in the present investigation, has also been reported by other workers in cows (Allcroft and Gordon, 1934; Lomba *et al.*, 1972 and Dutta, 1980). This decline in calcium may be attributed to excessive mammary uptake and the subsequent release into colostrum. The indications of reduced serum calcium levels on almost all the days of analyses in cows with placental retention as compared to cows with normal placental delivery are in agreement with Martinov (1964). However, Tsolov (1962) observed no relationship between reduced blood calcium levels and retained placenta in cows.

A disturbance in calcium homeostasis in cows retaining foetal membranes has not been unambiguously established. However, excessive mobilization of calcium from tail-bones in cows with retained foetal membranes may be a pointer towards this disturbance. Under physiological conditions estrogens and/or progesterone do not alter calcium storage in the bone or muscle tissue (Duckworth and Ellinger, 1956). It is not known whether an imbalance in estrogen/progesterone results into low serum calcium, or interfere with calcium uptake by myometrium in the bovine. However, the results of an *in vitro* study in rats revealed that diethylstilbesterol- a synthetic non-steroidal hormone, and progesterone are able to decrease calcium entry into the uterine cells which accounts for their inhibitory effect on the contractile activity (Batra and Bengtsson, 1978). Indispensibility of calcium for activating contractile proteins of muscle cells is well known (Weber *et al.*, 1963).

The reduction in inorganic phosphorus values with advancing parturition, has also been reported by Allcroft and Gordon (1974), Lomba *et al.* (1972) and Bostedt (1974) in cattle. A heavy demand for glucose and, therefore, its increased metabolism near parturition (Cornelius and Kaneko, 1963) and lactation may account for decrease in serum inorganic phosphorus values. The low serum inorganic phosphorus in cows with retained placentae may be explained on the basis of observation that retention of placentae is accompanied by reduction in blood glucose (Boiter *et al.* 1972; and Dutta, 1980). This reduction in blood glucose in cows with retained placentae may be due to its increased metabolism which involves inorganic phosphorus (Doxy, 1971).

It is evident that none of the four minerals except calcium, and inorganic phosphorus analysed in the present study, can be used to predict placental retention in cows.

REFERENCES

Allcroft, W.M. and Gordon, W. 1934. Biochem. J. **28**: 10007.

Batra, S. and Bengtsson, B. 1978. J. Physiol. **276**: 329.

Berger, E. and Marshall, J.M. 1961 Am. J. Physiol. **201**: 931.

Boiter, I., Mates, N. and Kadar, L. 1972. In: VII. Int. Congr. Anim. Reprod. and A.I. Munchen. I: 584.

Bostedt, H. 1974. Zentbt. Vet. Med. Beiheft. **20**: 172.

Cole, D.F. 1950. J. Endocr. **7**: 12.

Cornelius, C.E. and Kaneko, J. 1963. Clinical Biochemistry of Domestic Animals. Acad. Press, New York and London.

Doxy, D.L. 1971. Veterinary Clinical Pathology. Bailliere, Findall, London.

Duckworth and Ellinger, G.M. 1950. J. Endocr. **7**: 1.

Dutta, J.C. 1980. Some studies on the retained placenta in bovine. Dissertation submitted to Punjab Agricultural University, Ludhiana.

Fiske, C.H. and Subbarow, Y.J. 1925. J. Biol. Chem. **66**: 375. Cited D. Glick In: Methods of Biochemical Analysis, Vol. I Interscience Publishers Ltd. London, pp. 298.

Lomba, F., Chauvar, G. and Bienfet, V. 1972. Annls. Med. Vet. 116, Fasc. **6**: 563.

Martinov, V.G. 1964. Veterinariyna Moscow. 41: 78 (Vet. Bull. **34**: Abst. 3519).

Michell, A.R. and Noakes, D. 1980. In: Hormonal Regulation of Sodium Excretion. Ed. B. Lichardus, R.W. Schrier and J. Ponea, Elsevier. North Holland Biomed. Press. 1980. pp. 155.

Oser, B.L. 1976. In: Hawk's physiological Chemistry Ed. Bernard, L. Oser. Tata, McGraw-Hill Publishing Co. Ltd. New Delhi pp. 1141.

Tsolov, V. 1962. Vet. Bull **33**: Abst. 1780.

Weber, A. and Herz, R. 1963. J. Biol. Chem. **238**: 599.

Webster, Jr. W.W. 1962. Am. J. Clin. Path. **37**: 330.

Wilson, G.D.A., Hunter, J.T., Derric, G.H., Aitken, W.M. and Kronfeld, D.S. 1977. J. Dairy Sci. **60**: 111.

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Serum Protein Bound Iodine, Alkaline Phosphatase And Peroxidase In Buffalo Calves From Birth To Sexual Maturity

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ABSTRACT

The serum protein bound iodine (PBI), alkaline phosphatase (AKP) and peroxidase, three indicators of thyroid activity, were estimated in farm born, weaned at birth, male and female Surti buffalo calves at different stages from birth to the average age of sexual maturity. PBI, AKP and peroxidase showed marked increase after colostrum feeding. The variation from birth to sexual maturity was significant for all three estimates. The PBI and AKP showed significant decrease towards puberty and sexual maturity but the peroxidase behaved in the opposite way. The sex difference was non significant for all three estimates.

* * *

Measurement of serum protein bound iodine (PBI) is a reliable quantitative index of thyroid activity (Man, et al 1951). The enzyme thyroid peroxidase is a hemoprotein in nature and it is involved in the iodination of thyroxyl residues in thyroglobulin, to give rise to thyroxine (Taurog, 1970). Vadodaria et al (1978) has estimated serum peroxidase as an indicator of thyroid activity.

The present study was an attempt to know these estimates by following the same calves from birth to the average age of sexual maturity.

Materials and Methods

The study was conducted for three years at the Reproductive Biology Research Unit, Gujarat Agricultural University, Anand, India. On 23 Surti buffalo calves (9 males and 14 females) weaned at birth and fed colostrum 15-30 minutes after birth. The age at puberty in male calves (appearance of spermatozoa in penile discharge) was 452 days (15.07 months). The average age of sexual maturity in male (first ejaculate with full protrusion of penis) and female (occurrence of first oestrus) calves respectively was by 520 (17.33 months) and 480 (16.00 months) days. The blood was sampled by tapping the jugular vein, serum was separated and stored at −20°C until utilized. The schedule of blood sampling is as shown in Fig 1a (birth to 520 days).

The sampling stages from birth to 520 days in male calves were grouped into four consecutive periods namely; P1-neonate to prepuberal (birth to 100 days), P_2-prepuberal (125 to 235 days), P_3-puberal (300 to 445 days-P'_3) and P_4-postpuberal (460 to 520 days-P_4'). The situation in female calves remained the same except that P_3 ended at 415 days and P_4 ended at 480 days. The blood serum was sampled to estimate PBI, AKP and peroxidase by the methods of

TABLE 1 Analysis of Variance for stage, period and stage within period variation.

Characteristic		Source			
		Stage	Period	Stage/period	Error
Protein Bound Iodine	M	14.48* (32)	81.26* (3)	7.57 (29)	9.42 (153)
	F	22.76** (28)	108.05** (3)	12.53 (25)	10.17 (163)
Alkaline Phosphatase	M	1758.09**(32)	11536.77**(3)	746.50**29)	277.96(169)
	F	1600.25**(28)	5066.54**(3)	1184.29**(25)	285.19(215)
Peroxidase	M	39.93*(32)	64.96(3)	37.35*(29)	19.87(161)
	F	38.85**(28)	195.40**(3)	20.18 (25)	20.32(208)

* $P > 0.05$ ** $P > 0.01$

Note: Figures in the parenthesis indicate degrees of freedom. The error term for period was stage/period. M-Male calves, F-Female calves.

Acland (1957), King & Armstrong (1934), George (1953) and Machly (1954). Statistical analysis was done separately for male and female calves from birth to sexual maturity, to know variations due to stage or periods (Steel & Torrie, 1960). When the variations were significant then the critical difference was calculated. Pooled data from male and female calves were analysed to know the differences between periods, between sexes, between stages within period and the sex period interaction. The unequal number of observations at different stages were either due to involvement of calves born during different phases of experimental period or due to insufficient quantity of serum.

Results and Discussion

The levels of serum PBI, AKP and peroxidase found at different stages from birth to maturity in both the sexes of calves are given in Figuer 1a,2a, and 3a respectively. The means for different periods are presented in Figure 1b, 2b and 3b. The results of the statistical analysis are given in Table 1 and 2.

The *serum PBI* showed a clear increase (Fig. 1a) from its level at birth before colostrum feeding (AB) to 4 hr. after feeding colostrum (4 hr AFC). This agrees with the findings of Lewis and Ralston (1953b) in calves. This immediate increase in thyroid activity might be essential for better thermoregulation

TABLE 2 Analysis of variance for the pooled data

Characteristic	Source				
	Sex	Period	Sex × Period	Stage/Period	Error
Protein bound iodine	2.61(1)	143.37**(3)	3.08(3)	18.16*(23)	10.02(308)
Alkaline phosphatase	719.19(1)	12759.99**(3)	1436.16**(3)	2077.76**(23)	293.16(370)
Peroxidase	8.53(1)	265.20**(3)	94.47(3)	36.95(23)	21.33(360)

* $P > 0.05$ ** $P > 0.01$

Note: Figures in the parenthesis indicate degrees of freedom. The error term for period was stage/period and for others it is error.

required for the survival of the young (Ganong, 1967). The PBI level showed significant change (Table 1) from birth to sexual maturity (Fig. 1a) in male ($P<0.05$) and female ($P<0.01$) calves. It changed from 3.16 to 10.62 mcg% in male and 3.45 to 11.35 mcg% in female calves. Though there was no significant difference between many of the consecutive stages, as indicated by the critical difference test, the tendency towards reduction in the level with increasing age was there (Fig. 1a). The mean for P_1 to P_4 were 7.30, 6.10, 4.86 and 4.52 mcg% respectively in male calves. In female calves the respective values were 7.78, 5.81, 4.83 and 4.89 mcg% (Fig. 1.b). So in both sexes the serum PBI decreased from early age towards puberty and tended to maintain or slightly increase towards sexual maturity. The decreasing trend of PBI up to puberty, recorded in the present study, agrees with the studies of Lewis and Ralston (1953 a,b) and Gill, et al (1966) on the effect of age on PBI level, but does not agree with the observation of Omar et al (1973) in buffaloes.

The sex difference in PBI (for the data considering both chronology and periods) was non significant (Tab. 2). The mean recorded for male and female calves were 6.40 and 6.57 mcg% respectively. This result agrees with that of thyroid activity in Zebu and crosshred cattle (Gill, et al 1966).

The *serum AKI* showed significant ($P<0.01$) decline from birth to maturity in calves of both sexes (Tab. 1 and 2). Its level in male calves at birth was 54.08 K.A.U.% and increased to 87.44 K.A.U% at 4hr. AFC. In female calves the AKP at these two stages was 38.61 and 80.31 K.A.U.% respcetively. In both Sexes the AKP level declined subsequent to 4 hr. after feeding colostrum. The decrease in AKP level with age becomes more clear when the means for different periods are observed (Fig. 2b). In male calves the mean AKP for P_1, P_2, P_3 and P_4 was 44.39, 19.77, 17.55 and 14.33 K.A.U.% respectively. For the same periods in females the levels were 34.63, 24.23, 16.33 and 16.16 K.A.U.%. The reduction in serum AKP level recorded in the present study agrees with the reports in male and (Roussel & Stalicup, 1966), female calves (Agergaard & Larsen, 1973). However, Roubicek & Ray (1974) and Pandiya et al (1977) have recorded increase in serum AKP level with age in cattle. The higher AKP found in early age in our study might he due to higher osteoblast activity for bone formation and to higher metabolic rate. AKP activity is also reflective of growth hormone secretion (Freeland & Szepest, 1971), Concurrent with higher serum AKP activity, higher serum calcium and inorganic phosphorus has also recorded (in another parallel study in the some calves (Devaraj, 1982). Further, the periods of higher serum AKP activity showed higher serum PBI also.

The overall average serum AKP for male (30.13 K.A.U.%) was slightly higher than for female (27.42 K.A.U.%) calves and they grow slightly faster compared with females. However, the sex difference was non-significant for AKP levels (Tab. 2). This is in agreement with the findings of Kaker et al (1969) but not with Singh, et al (1972) in buffalo calves. Roubicek and Ray (1974) have also recorded sex difference in cattle.

The *serum peroxidase* levels from birth to maturity (Fig. 3a) changed significantly in both the sex (Tab. 1). Its level ranged from 1.43 to 13.28 O.D./ml/10 min./30°C in male and 2.55 to 11.46

O.D./ml. In female calves. The increase in its level from values at birth to 4 hr. AFC was more clear in male calves (7.42 to 10.47 O.D./ml) than in female calves (4.79 to 5.43 O.D./ml Fig. 3a). The peroxidase level from 3 days onwards up to the completion of P_1 was almost constant and then showed an increase towards puberty. Its means for P_1, P_2, P_3 and P_4 in male calves was 3.70, 4.57, 6.49 and 4.20 OD/ml. The level for these periods in female calves were 3.50, 5.18, 6.84 and 7.53 O.D/ml (Fig 3b). So its increase up to maturity was clear in female calves but in males it was up to puberty only. The trend with regard to peroxidase activity was opposite to that of AKP and PBI which were decreasing. Though, there are no comparable studies on serum peroxidase, Gangawar and Untawala (1971) recorded low leucocytic peroxidase activity in children and young ones of rat, rabbit and pig, compared with their respective adults. The sex and sex period variation was nonsignificant (Table 1). The overall means for male and female calves were 4.63 and 4.93 O.D/ml. respectively. These means are higher than those recorded in Surti buffalo heifers (Vadodaria et al 1978).

In the present study feeding of colostrum about 15 minutes after birth appears to have an important biochemical bearing as shown by the increase in serum levels of PBI, AKP and peroxidase. The change might help for better thermoregulation and higher metabolic activity during early age. The overall trend of PBI and AKP paralleled together and their levels decreased with advancing age till sexual maturity. The levels of enzyme peroxidase followed a trend opposite to that of AKP and PBI. The data suggest that the AKP levels might be used as an index to predict the advancement of sexual maturity.

Sustained levels of PBI and AKP might be helpful for better growth of long bones and consequently better overall growth of the calves. This assumption is supported by the fact that the average height of 3 to $3\frac{1}{2}$ year old village based heifers (purchased for some other experiment) was only 112.4 cms at withers, with a mean body weight of 195 kg.; whereas the female calves included in the present study had an average height of 126.5 cm at withers, with a mean body weight of 205 kg, at 480 days of age (i.e. at sexual maturity), which is impressively better. The male calves in the present study averaged 229 kg. at the time of sexual maturity (520 days of age). As these attained adequate body weight and sexual maturity by 15-17 months of age, the values estimated may be considered as norms and thus the said estimates are mapped out from birth to sexual maturity in this breed.

REFERENCES

Acland, J.D. (1957). The estimation of serum protein bound iodine by alkaline incineration. Biochem. J. **66**: 177-188.

Agergaard, N. and Larsen, B. (1973). Bovine plasma alkaline phosphatase activity in relation to age, J substance and beta lactoglobulin phenotypes. In Aarsberetning Inst. for sterilitcis forsking, Copenhagen. pp. 81-96 (Vet. Bull. **44**: 1332).

Devaraj, M. (1982). Blood serum profile in calves and postpartum buffaloes (Surti breed) with associated peridata related to reproductive efficiency. Ph.D. thesis Gujarat Agricultural University, Anand Campus, Anand, India.

Gangawar, I.C. and Untawala, G.G. (1971). Peroxidase of leucocytes in different animals and man. Indian J. Physiol. Pharmac. **15**: 15-19.

Ganong, W.P. (1967). Review of Medical physiology. 3rd Edn. 1 Lange Medical Publications, Maruzen company Ltd.

George, P. (1953). Intermediate compounds formation with peroxidase and strong oxidising agents. J. Biol. Chem. **201**: 413-426.

Gill, R.S. Rattan, P.J.S., Singh, R. and Ananthakrishnan, C.P. (1966). Plasma protein bound iodine level in zebu and cross-bred animals. Indian J. Dairy Sci. **19**: 42-44.

Kaker, M.L., Chakravorthy, S.C. and Mishra, B. (1969). Serum engymes in Indian Livestock. 2. Phosphatase activity in blood serum of Haryana cattle and Murrah buffaloes. J. Res. Ludhiana. **6**: 394-399.

King, E.J. and Armstrong, A.R. (1934). Can. Med. Assoc. J. **31**: 37 cited by varley, H. (1963). Practical clinical biochemistry. 3rd Edn. Intersecience books, Inc, New York.

Lewis, R.C. and Ralstom, N.P. (1953). Protein bound iodine levels in dairy cattle plasma. J. Dairy Sci. **36**: 33-38.

Lewis, R.C. and Ralston, N.P. (1953b). Changes in the plasma levels of protein bound iodine in the young calf. J. Dairy Sci. **36**: 363-367.

Machly, A.C. (1954). Determination of peroxidase activity. Methods of biochemical analysis. Vol. **1**: 385 (D. Glick, Ed.) Interscience publishers, Inc., New York.

Man, E.B., Kydd, D.M. and Peters, J.P. (1951). Butanol extractable iodine of serum. J. Clin, Invest. **30**: 531.

Omer, M.A., Valfre, F., Macri, A. and persechino, A. (1973). Vit. A, calcium, phosphorus, PBI, potassium and Zinc content of serum of buffaloes. Acta Medica Veterinaria. **19**: 357-367 (Vet. Bull. **45**: 486).

Pandiya, S.C., Dwarakanath, P.M. and Rathor, S.S. (1977). Studies on serum calcium, inorganic phosphorus and alkaline phosphatase in dairy cattle, Indian Vet. J. **54**: 130-133.

Roubicek, C.B. and Ray, D.E. (1974). Serum alkaline phosphatase in unsupplemental range cattle. J. Anim. Sci. **38**: 1185-1189.

Roussel, J.R. and Stallcup, O.T. (1966). Influence of age and season on phosphatase and transaminase activities in blood serum of bulls. Am. J. Vet. Res, **27**: 1521-1527.

Singh, A.P., Joshi, H.C. and Singh, R. (1972). Studies on certain blood constituents in cattle and buffaloes. Vet. J. **49**: 473-477.

Stell, R.G.D. and Torrie, J.H. (1960). Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York.

Taurog, A. (1970). Thyroid peroxidase and thyroxine blosynthesis. In Recent progress in hormoneresearch (E.B. Astwood, Ed.).

Vadodaria, V.P.; Janakiraman, K. and Buch, N.C. (1978). Serum Enzymes to reflect thyroid activity in Surti heifers in relation to estrous cycle and breeding periods. Ind. J. Physiol. and Allied Sci. Vol. 32, No. 3, p. 108-115.

IJAR 5: 1: 54-58 1984

Total Protein Concentration In Uterine Secretions Of Buffaloes During Certain Reproductive Phases

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ABSTRACT

The total protein concentration in the uterine secretions of buffaloes was investigated during pro-oestrus, oestrus, dioestrus and early pregnancy. The protein concentration was found to vary with the phase of the oestrous cycle. The concentration recorded was maximum during dioestrus. From the pressent study it is suggested that progesterone favour protein release into uterine secretions. The higher concentration in dioestrus may be to nourish the embryo if fertile coitus has taken place. In early pregnancy the protein concentration was lower than in dioestrus which may be due to the probably lower levels of progesterone during early pregnancy and its absorption by the conceptus for the development.

* * *

The importance of uterine secretions in female reproductive system is well recognised. In recent years, there has been an increasing interest in defining the biochemical nature of the uterine environment, though efforts to this end have heen hindered by the small quantities of the fluid available and the difficulty in obtaining the fluid. It is believed that intrauterine environment exerts a pronounced effect on sperm capacitation and embryonic development. There is evidence that uterine protein secretions are essential for embryonic development beyond blastocyst stage in cows (Bazer 1975). A knowledge on the concentration of total protein in uterine secretions during different phases of the physiology of reproduction would aid in the control of infertility problems. Hence, a study was made in buffaloes which are contributing 60 per cent of the country's milk production.

Materials and Methods

Genitalia from 45 non pregnant and 15 early pregnant (25-30 days) buffalo cows were collected from the slaughter house and they were transported to the laboratory in an ice box. The nonpregnant uteri were classified into pro-oestrus, oestrus and dioestrus based on the gross morphology of the ovaries as per the techniques of Choudary *et al.* (1968) for follicular size and Abul Fadle *et al.* (1974) for the corpus luteum size. The pregnant uteri were grouped as early pregnancy based on the biometry of the conceptus as per the technique of Arthur (1968).

The method of Olds and VanDemark (1957) was adopted for the collection of uterine secretions, with a slight modification i.e. a wooden roller pin was used in the collection technique. From the pregnant uterus uterine secretion was collected in the same manner after the

TABLE 1 Total protein concentration (g/100 ml) in uterine secretions of buffaloes during certain phases of reproduction

Reproductive phases	n	Mean	S.E.	Range
Pro-oestrus	15	8.04	0.29	5.90 to 10.00
Oestrus	15	4.09	0.55	1.00 to 9.10
Dioestrus	15	12.03	0.54	8.50 to 15.00
Early Pregnancy	15	8.15	0.67	4.80 to 12.20
'F' Observed	37.39**	(d.f. 56)		

Statistical comparison	Difference value	LSDT value	Inference
Pro-oestrus to oestrus	3.95	1.04	**
Pro-oestrus to Dioestrus	3.99	1.04	**
Oestrus to Dioestrus	7.94	1.04	**
Pro-oestrus to Early pregnancy	0.11	0.78	NS
Oestrus to early pregnancy	4.16	1.04	**
Dioestrus to Early pregnancy	3.88	1.04	**

n = no. of samples
S.E. = Standard error
d.f. = Degree of freedom
** = Significant at $P>0.01$
NS = Nonsignificant at $P>0.05$
LSDT = Least Square Difference Test

removal of conceptus by making a transverse incision dorsally just anterior to the osinternus and lifting the uterine horns from the tubal end for the escape of the conceptus along with the fetal sacs and after a ligature was applied anterior to the incision. Those uteri which showed placental formation were discarded. The samples which contained cloudy flakes and or blood tinge were discarded. Immediately after collection the samples were stored at $-4°C$ until they were analyzed for total protein by Biuret method as described by Wooton (1974). Statistical analysis of the data was done as per the method of Steel and Torrie (1960).

Results

There was a significant ($P<0.01$) variation between the phases of oestrous cycle studied. Its concentration was highest in dioestrus and lowest in oestrus. In early pregnancy its concentration was significantly ($P<0.01$) lower than dioestrus and higher than oestrus. Mean values are recorded in the Table 1 and Fig. 1.

Discussion

The values recorded in this study during oestrus is in agreement with Schultz *et al.* (1971) and Lamothe *et al.* (1972) in cows. The values of the other phases varied marginally, perhaps it could be due to species variation. There was a significant increase in the protein concentration during luteal phase than in follicular phase in ewe, cow, sow and mare (Heap 1962; Schultz *et al.* 1971; Chen *et al.* 1973; Zavy and Bazer 1978). A similar trend has been noticed in buffaloes by Pahwa *et al.* 1980, and also in the present study, higher protein

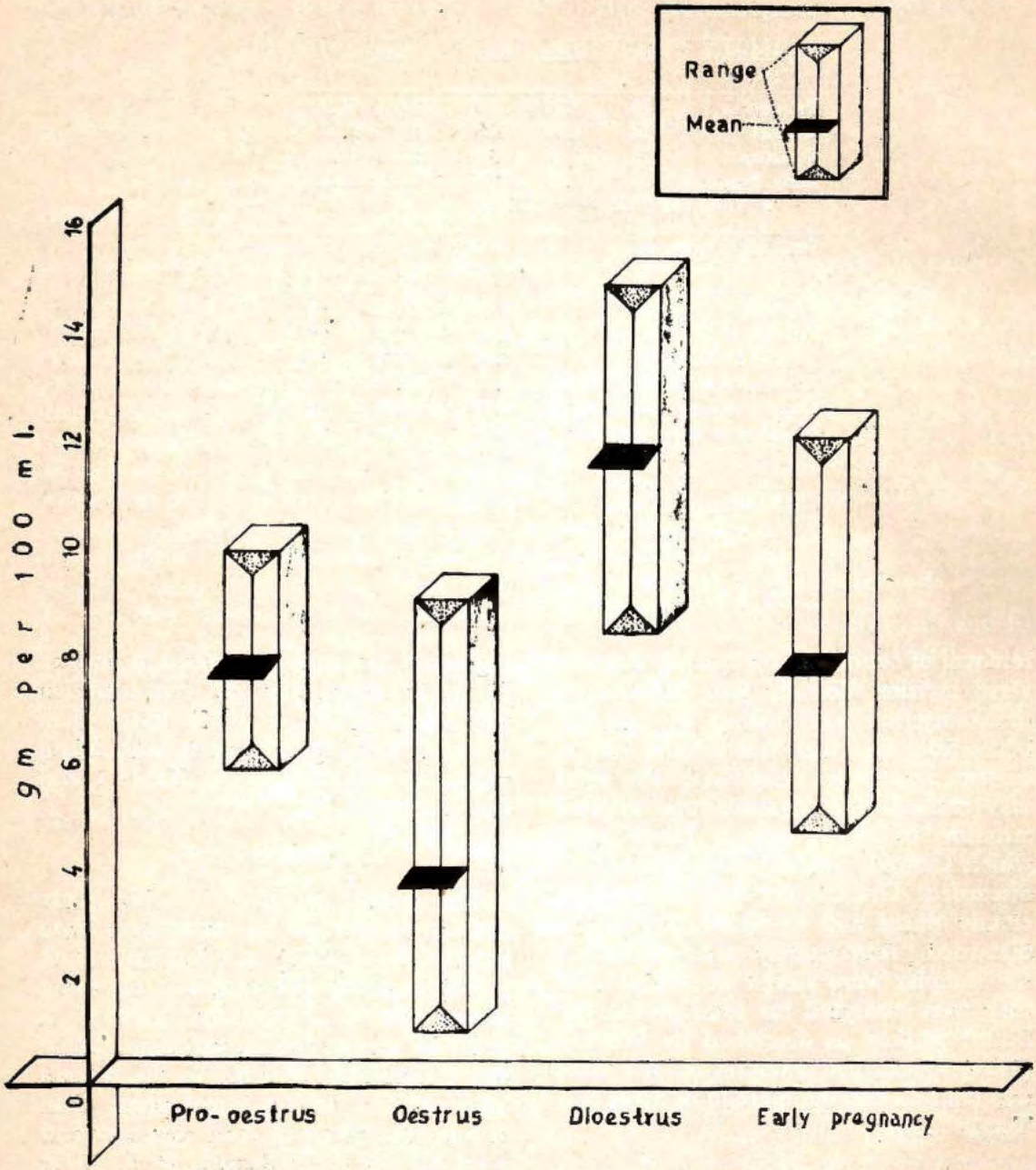

FIG 1 TOTAL PROTEIN CONCENTRATION IN UTERINE SECRETION OF BUFFALOES DURING PRO-OESTRUS, OESTRUS, DIOESTRUS AND EARLY PREGNANCY

content during the dioestrus in the present study may be attributed to high blood progesterone level during this phase. Knight *et al.* (1973) has demonstrated that progesterone controls porcine uterine secretions.

In early pregnancy there was a significant decrease ($P<0.01$) in the concentration of total protein than in dioestrus.

Progesterone therapy increases protein concentration in uterine secretions of sows (Kovalenko 1972). Thorburn and Schneider (1972) are of the opinion that progesterone concentration during first forty days of pregnancy decreases slightly from the level seen during dioestrus. The conceptus 'Protects' the corpus luteum during pregnancy. The period over which it influences to establish the corpus luteum is short in pig and it takes 50 days in sheep (Moor 1968), during which time sufficient Luteotrophic hormone to maintain corpus luteum is absent (Heap *et al.* 1973). In cows it is suggested that Luteinizing hormone is the major component of Luteotrophic hormone complex, which stimulate progesterone synthesis by the corpus luteum to optimum level later a period after conception. Hence, the lower concentration noticed in the present study during early pregnancy may be due to the lower progesterone level.

Proteins in uterine secretion is essential for nidation in rats (Schinozuka 1980), better survival of embryos in sow (Kovalenko 1972) and development of conceptus in buffaloes (Pahwa *et al.* 1980). Ayalon (1978) recorded significantly lower levels of protein in early pregnancy than in dioestrus cows which were unbred. In sow, proteins serve in transporting water-insoluble nutrients into the conceptus from uterine millieu (Adams *et al.* 1981). These findings suggest that developing conceptus utilizes the protein present in the uterine secretions. This may also be a probable reason for the lower concentration of total protein observed in the present study during early pregnancy than in dioestrus apart from the lower concentration of blood progesterone during early pregnancy.

REFERENCES

Abul Fadle, N.S., Fahmy, M.F.A. and Shafey, S.M. (1974). Histological studies on the ovaries of Egyptian buffaloes with special reference to the corpus luteum. Bbl. vet. Med. **21**(A): 581-591.

Adams, K.L., Baeer, F.W. and Roberts R.M. (1981). Progesterone induced secretion of a retinol binding protein in the pig uterus. J. Reprod. Fert. **62**: 39-47.

Arthur (1968). *Wright's Veterinary Obstetrics*. 3rd Ed. The English language book society, London pp. 60-62.

Ayalon, N. (1978). A review of Embryonic mortality in cattle J. Reprod. Fert. **54**: 483-493.

Bazer. F.W. (1975). Uterine protein secretions relationship to development of the conceptus. J. Anim. Sci. **41**: 1376-1382.

Chen, T.T., Baeer, F.W., Cetorelli, J., Pollard, W.E., and Roberts, R.M. (1973). Purification and properties of progesterone induced basic glycoprotein from the uterine fluids of pigs. J. biol. Chem. **248**: 8560-8566.

Choudary, J.B., Gier, H.T. and Marion, G.B. (1968). Cyclic changes in bovine vesicular follicles. J. Ani.Sci. **27** 468-471.

Heap, R.B. (1962). Some chemical constituents of uterine washings a method of analysis with result from various species. J. Endocr. **24**: 367-378.

Heap, R.B., Perry, J.S. and Challis, J.R.G. (1973). Hormonal maintenance of pregnancy, *Hand Book of Physiology*. Section-7, Endocrinology, Vol. II, Part 2. Ed. Roy. 0, Greep., American Physiological society, Washington, pp. 217-260.

Kovalenko, V.F., (1972). Histotrophic nutrition and Embryo mortality in pigs, In VII Int. Congress ° n Anim. Reprod. and A.I. II., 1185.

Knight, J.W., Bazer, F.W. and Wallace. H.D. (1973). Hormonal regulation of porcine uterine protein secretion. J. Anim. Sci. **36:** 546-553.

Lamothe, P., Guay, P., Ibrahim, M. and Tremblay, A. (1972). Total proteins and their fractions in the serum and uterine secretions of cows with nonspecific infertility. Can. J. Comp. Med. **36:** 94-98.

Moor, R.M. (1968). Effect of embryo on corpus luteum function. J. Anim. Sci. **27:** 97-128.

Olds, D. and VanDemark, N.L. (1957). Composition of luminal fluids in bovine female genitalia. Fert. Steril. **8:** 345-354.

Pahwa, G.S., Kumar, D. and Pandey, R.S. (1980). Biochemical analysis of uterine fluid of the buffala (Bubalus bubalis) Zbl. Vet. Med. **27** (A): 81-84.

Schinozuka, M (1980). Some aspects of nidus-endometrial relationships with special reference to the uterus specific proteins that appears in the endometrial tissue in the initial stage of implantation. Japanese, J. Fert. Steril. **25:** 62-72.

Schultz, R.H, Fahming, M.L. and Graham, E.F. (1971). A chemical study of uterine fluid and blood serum of normal cows during the oestrous cycle. J. Reprod. Fert. **27:** 355-367.

Steel, G.D. and Torrie, (1960). *Principles and Procedures of Statistics*. McGraw-Hill Book Company Ltd., London.

Thorburn, G.D. and Schneider, W. (1972). The progesterone concentration in the plasma of the goat during the oestrus cycle and pregnancy. J. Endocr. **52:** 23-36.

Wooton, I.D.P. (1974). *Microanalysis in medical biochemistry* 5th Ed. Churchill Livingstone, Edinburgh.

Zavy, M.T. and Bazer, F.W. (1978). A nonsurgical technique for the collection of uterine fluid from the mare. J. Anim. Sci. **47:** 672-676.

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Protein Bound Iodine During Pregnancy In Surti Buffalo

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ABSTRACT

Blood serum PBI was estimated from pregnant buffaloes for successive three pregnancies. The average PBI in pregnant buffalo covering 672 samples was 5.97 mcg/100 ml., which was higher compared to non-pregnant. The concentration of PBI was considerably high during early gestation compared to mid and late gestation. After Day 50 of gestation the trend was declining till parturition. Towards the term, last 15 days; a steap fall in the estimate was noticed, which again increased soon after calving. Post-partum performance was not adequate in those animals which had lower PBI during pregnancy.

* * *

Measurement of serum PBI, as 90% of T4 is bound with protein, is a reliable quantitative index for evaluating thyroid activity (Man. *et. al.* 1951). Thyroid hormone also plays a role in the physiology of reproduction. It is linked with that part of adrenal cortex which has influence on the various reproductive processes (Maqsood, 1954). There is an interrelationship between thyroid, adrenal and gonads.

Materials and Methods

The experiment included total of 24 pregnancies (Surti buffaloes). These animals underwent 3 successive pregnancies. Each pregnancy had 27 stages, from fertile heat to parturition. Blood collection was done from jugular vein, serum was separated and stored at −20°C with merthiolate as preservative till analysed. Estimation of PBI was done by the standard alkaline ashing method of Acland (1957). The data were analysed statistically by the model of Randomised Block Design as stated by Panse and Sukhatme (1978). For detail analysis these 27 stages were grouped into 7 phases (Table 1) according to physiological significance.

TABLE 1 Details of pregnancy phases

Phases	Physiological stage
One	Fertile heat
Two	Day 2 to Day 10 (Ovulation, Fertilieztion and Shedding of Zona pelucida).
Three	Day 15 to Day 50 (Placentation and Implantation).
Four	Day 65 to Day 125 (Foetation and organogenesis).
Five	Day 155 to Day 275 (Development of foetus).
Six	Last 15 days of to parturition (Development of udder, Teat engorgement, Uterine contraction, initiation of labour Pain and Parturition).
Seven	2hr Post-partum.

Results

The average concentration of PBI calculated from 672 samples covering three pregnancies was 5.97 mcg/100 ml.

TABLE 2 Serum BBI (mcg/100 ml) Mean ±SE in pregnant buffalo

Stage/day	Heat	2nd	3rd	5th	10th	15th	20th	25th	30th
Parity I	5.02	9.11	6.39	7.55	10.64	6.60	4.50	6.92	8.77
	±0.45	0.70	0.77	0.61	0.64	0.36	0.49	0.89	0.73
II	5.67	7.68	6.32	9.44	5.83	7.21	7.40	10.65	6.34
	±0.72	0.58	0.83	0.90	0.86	1.41	0.88	1.47	0.95
III	5.59	8.13	6.71	7.83	9.28	7.28	7.63	7.46	6.40
	±0.73	0.81	0.12	0.50	0.60	0.36	0.48	0.72	0.51

Stage	35th	50th	65th	80th	95th	125th	155th	185th	215th
Parity I	6.48	5.69	5.74	6.88	5.78	5.49	7.73	7.33	6.55
	±0.79	0.62	0.60	0.68	0.92	0.64	0.78	0.88	0.64
II	7.37	8.22	6.92	7.22	7.16	5.72	4.03	4.84	5.89
	±1.68	1.17	0.98	1.12	0.78	0.76	0.53	0.62	1.76
III	8.19	8.58	7.28	7.67	8.32	6.55	7.45	6.93	7.75
	±0.62	0.64	0.75	0.48	0.91	0.57	0.37	0.50	0.71

Stage	245th	260th	270th	275th	− 15	− 10	− 5	AP.*	PP.**
Parity I	5.86	5.88	6.77	5.91	5.48	5.48	5.19	3.63	5.29
	±0.78	0.83	1.20	0.60	0.61	0.69	0.83	0.39	0.40
II	4.20	4.88	4.82	5.73	4.15	3.22	4.42	6.95	5.98
	±0.47	0.75	0.74	0.82	0.75	0.49	0.80	1.86	1.85
III	6.49	8.01	7.52	7.22	8.56	8.17	6.76	5.34	5.66
	±0.46	0.58	0.50	0.30	0.79	0.71	0.81	0.49	0.87

* AP = 2hr Antepartum ** PP = 2hr. Post-'partum.

The average for first, second and third pregnancies were 6.39, 5.99 and 6.98 mcg/100 ml respectively. Estimated levels for all the 27 stages have been given in Table 2.

Graphical presentation in the figure (Fig. 1a) represents the mean for three pregnancies against 27 stages. It is clear that oscillating higher levels were observed during early gestation compared to late gestation. Appreciable decrease in the estimate has been noticed after 80th Day of gestation. A considerable increase was recorded soon after calving.

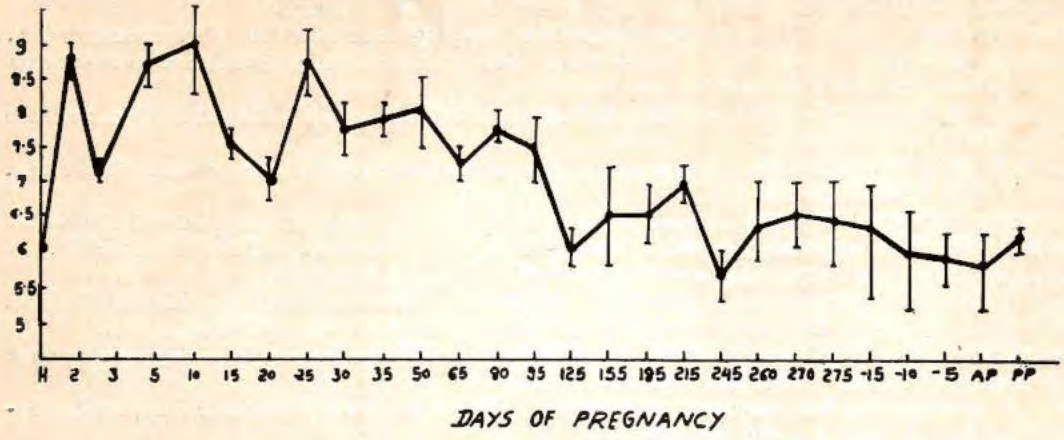
Phasewise analysis (Presented as bar diagram figure 1b) revealed that PBI estimate increased during period of ovulation, fertilization and release of blastocyst by shedding the zona pellucida (Phase-2). Then it decreased till 6th phase (parturition).

Discussion

PBI got elevated during pregnancy compared to previous cycles of the same animals (5.46 mcg% Pathak, 1983). Work done by Kiesel and Burn (1960); Artner and Golob (1964); Kaneko and Corneleus (1970) and Hussan and Alakkan (1960) for selected stage of gestation also reveals higher PBI in pregnant cattle. This increase may be attributed to increased metabolic rate during pregnancy. Marine (1917), one of the pioneer worker on thyroid gland, named this as "Workhypertrophy".

Undulating higher levels were present up to Day 50 of gestation, a period by which placental establishment completes in bovine; reflects its demand and synthesis (Raizada and Pandey 1984). There after, the estimate declined. This may be due to utilization by growing foetus by getting maternal PBI transferred through

Fig. 1a SERUM PBI (Mean ± SE) AGAINST THE 27 STAGES OF PREGNANCY.



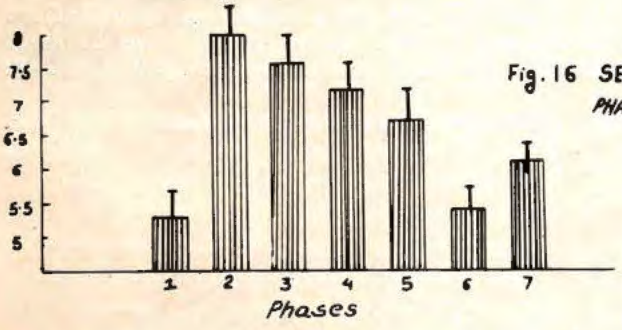


Fig. 1b SERUM PBI (Mean ± SE) AGAINST THE 7 PHASES OF PREGNANCY.

placenta as needed. Such possibility is reported by Warner (1962).

The second phase of decrease starts from 270th Day of gestation, which is about 22% of the level for early gestation. This fall in maternal PBI may be due to its involvement and utilization for development of mammary gland and milk synthesis. Similar type of trend in serum PBI for late gestation have also been reported in cattle by Lewis and Ralston (1953) and Robertson *et. al.* (1957). Their explanation for the decrease was the utilization of it for milk synthesis. Work of Maqsood, (1954) and Pipes *et. al.* (1958) has proved the requirement and influence of thyroid hormone on milk secretion.

A detailed look into the individual animal in our study indicates that a delay in the occurrence of first heat and service period during postpartum was noticed in those animals which had lower PBI in the circulating blood during second half of gestation. This leads to an understanding that supplementation of iodine during late gestation as one among the managerial factor may help to improve the reproductive performance during postpartum period.

REFERENCES

Acland, J.D. (1957). The estimation of serum protein bound iodine by alkaline incineration. Biochem. J. **66**: 177-188.

Artner, J. and Golob, E. (1964). Thyroid function in pregnancy (Zum, Problem der. Schilddruesenfunldi in der Schwangerschaft) Geburtsh and Franenh. Am. J. Gyn. & obs. **24**: 325 (Abstract).

Ganong, W.F. (1967). Review of medical Physiology, 3rd Edn. Lange Medical Publications, Maruzen company Ltd.

Hussan, A. and AlSakkan (1980). Plasma protein bound iodine and lactational performance of crossbred cows during pregnancy. Indian J. Dairy Sci. **33**: 304-309.

Kaneko, J.J. and Corneleus, C.E. (1970). Clinical Biochemistry of domestic animals. Vol. I and 2nd Edn. Academic press, New York. pp. 313-394.

Kiesel, G.K. and Burns, M.J. (1960). A preliminary report on the Serum protein bound iodine in dairy cattles. Am. J. Vet. Res. **21**: 226-229.

Lewis, R.C. and Ralston, N.P. (1953). Protein bound iodine levels in dairy cattle plasma. J. Dairy Sci. **36**: 33-38.

Man, E.B., Kydd, D.M. and Peters, J.P. (1951). Butanol extractable iodine of serum. J. clin, invest. **30**: 531.

Maqsood, M. (1954). Role of thyroid hormone in reproduction. Ind. Vet. J. **31**: 23-30.

Marine, D. (1917). The thyroid gland in gynaecology and obstectics. Surg. Gynec. and Obst. **25**: 272 (Abstract).

Panse, V.G. and Sukhatme, P.V. (1978). Statistical method for agricultural workers. ICAR New Delhi.

Pathak, M.M. (1983). Ph.D. Thesis. Blood serum profile in Pregnant buffaloes (Surti breed) during different stages of pregnancy. Gujarat Agricultural University, Anand.

Pipes, G.W. Premachandra, B.N. and Turner, C.W. (1958). Effect of estrogen and progesterone on thyroid function of cattle. J. Dairy Sci. **41**: 1387-1394.

Raizada, B.C. and Pandey, M.D. (1984). Temporal changes in buffalo embryo during early pregnancy (Abastrct). Proceedings of International symposium on foetal biology 20th Feb. 1984. Held at Mathura vet. college, Mathura.

Robertson, W.G., Lennon, H.D. Jr; Bailey, W.W. and Mixner J.P. (1957). Interrelationship among the plasma 17-Hydroxy corticosteroids plasma protein bound iodine levels and Ketosis in Dairy cattle. J. Dairy Sci. **40**: 732-736.

Warner, Sidney, C. (1962). "The Thyroid" 2nd Edn. A fundamental and clinical text. A Hoeber Medical book. Harper & Roa Publishers.

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Plasma Levels of Peripheral Prostagandin F2α In Buffalo Female Neonates.

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ABSTRACT

A marked difference in the Prostaglandin $F_2\alpha$ concentrations was observed in the suckling and non-suckling buffalo calves. The concentrations being higher in non-suckled calves.

* * *

The development of a highly sensitive method of radioimmunoassay had made it possible to measure the circulatory levels of prostaglandin $F_2\alpha$ in animals and to monitor the changes in the circulatory levels during growth and reproduction. How these pharmacological agents interact with the genetic and nutritional factors and control growth process was still unexplored area of research. Since no information was available in the literature regarding the circulatory levels of prostaglandin $F_2\alpha$ in the peripheral blood plasma during neonatal period, influenced by non suckling response in buffalo heifers, the present study was undertaken to measure the normal circulatory levels of prostaglandin $F_2\alpha$ during neonatal period in buffalo female heifers.

Materials and Methods

Murrah buffalo female calves of day 1 to day 3 of age were selected from the herd of National Dairy Research Institute for the study. The selected animals were kept in a loose housing system separately under the similar conditions of feeding and management. Blood sampling was done prior to feeding each day between 0800 hr and 1000 hr. Blood samples were drawn daily in the heparinized chilled glass stoppered tubes from the jugular vein with least undue stress to the animals on days 1, 2 and 3. The blood samples were kept in ice immediately after collection and the plasma was separated by centrifuging at 3000 rpm for 30 min. The plasma was stored at −15°C pending prostaglandin $F_2\alpha$ analysis.

Radioimmunoassay of prostaglandin $F_2\alpha$ in blood plasma.

The prostaglandin $F_2\alpha$ in the peripheral blood plasma was estimated by the procedure of Hixon, et al. (1973) with some modifications (Table 1). Blood plasma (0.3 ml) was taken in clean and sterilized glass tubes of 12 × 100 mm (cornings) and assayed without extraction in duplicate. The samples were incubated at room temperature (30°C) with 0·1 ml of a specific prostaglandin $F_2\alpha$ antiserum dilution (1:1000. Thirty min later, 0.1 ml of the labelled prostaglandin $F_2\alpha$ about 4000 cpm) was added to each sample tube and incubated at room temperature. The standard tubes contained 0.4 ml PBS buffer (pH 7.00), 0.1 ml of 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 pg/0.1 ml prostaglandin $F_2\alpha$ standard 0.1 ml specific antiserum and 0.1 ml labelled $PGF_2.\alpha$ The tubes were vortexed vigorously and then incubated at 4°C for

TABLE 1 Experimental conditions and validity of radioimmunoassay for the estimation of prostaglandin $F_2\alpha$ in blood plasma of buffaloes.

Experimental conditions	
Plasma volume	0.3 ml
Antibody dilution	1:1000 (Initial)
Tracer added to each tube	4000 cpm
Incubation volume	0.5 ml
Incubation temp.	+4°C
Incubation time	20 hr.
Seperating agent for bound and unbound hormone	Activated charcoal dextran
Volume of separating agent added	0.5 ml at +4°C
Centrifugation temp.	+4°C
Centrifugation time/speed	15 min/3000 rpm
Counting portion	Suppernatant (PPO) (POPOP) (Biosolv)
Cocktail added	5 ml
Counter used	Packard Tri carb

Validity			
Sensitivity		2 pg	
Intra-assay C.V.		8.50	
Inter-assay C.V.		12.37	
Recovery (pg)			
No. of replicates	$PGF_2\alpha$ added	$PGF_2\alpha$ recovered	C.V.
4	50	57.72±2.43	6.01
4	100	117.56±4.35	5.22
10	250	267.09±19.58	16.43
20	500	483.51±34.12	15.81
r = 0.9986 (P<0.01)			
b = 0.9375			
y = 21.4177			

±SEM = Standard error of mean
C.V. = Coefficient of variation
r = Coefficient of variation
b = regression
y = Dependent variable.

20 hr. Dextran charcoal 0.5 ml suspension was added in each tube at 4°C and the mixture was vortexed for 15 seconds. The tubes were centrifuged for 15 min at 3000 rpm at 4°C. The supernatant was transferred to the liquid scintillation in glass vials containing 5 ml. scintillation fluid with 10 per cent Blosolv. Counting was performed in a Packard Prias Tri-Carb Automatic Liquid Scientillation Counter, equipped with Tele Type Writer with a punch. The sensitivity, Intra and Inter assay of variation and recovery are given in Table 1. The specificity of antibody to prostaglandin $F_2\alpha$ was reported earlier (Shemesh *et al;* 1978).

Results and Discussion

TABLE 2 Plasma levels Prostaglandin $F_2\alpha$ (ng/ml) in buffalo female neonates.

Age	Weaned	Suckling
Day 1	2.64±0.50(18)	0.81±0.62(4)
Day 2	2.20±0.41(17)	0.58±0.26(4)
Day 3	2.74±0.67(16)	1.38±0.71(2)

Figures in parenthesis indicate number of animals.

The suckling and non suckling buffalo female calves had a marked difference in the prostaglandin $F_2\alpha$ concentrations being higher in non suckled calves. However, there were slight decrease on day 2 of birth in both the groups. The results of this study, seems quite interesting as the levels in neonates were quite high to the levels of prepubertal and peripubertal heifers (Jain, 1983). The higher level of prostaglandin $F_2\alpha$ in neonates may be indicative of fetal origin and a poor metabolic clearance rate in neonates. It is difficult to substantiate these findings as no work on the estimations of prostaglandin $F_2\alpha$ has been reported in dairy animals. However, Mitchell *et al.* (1978) did measure the $PGF_2\alpha$ levels from birth onwards in children and reported a similar pattern.

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REFERENCES

Hixon, J.E., Nadraja, R., Schechter, R.J. and Hansel, W. (1973). Prostaglandins **4**: 679.

Jain, G.C. (1983). Studies on prostaglandin $F^{3}\alpha$ and some reproductive hormones in blood plasma of buffalo heifers. Ph.D. Thesis, Kurukshetra University, Kurukshetra.

Mitchell, M.D., Flint, A.P., Bibby, J., Brunt, J., Arnold, J.M., Anderson, A.B.M. and Turnhull, A.C. (1978). J. Clin. Endocr. Metab. **46**: 947.

Shemesh, M., Ailenberg, M., Mitaguer, F., Ayalon, N. and Hansl, W. (1978). Biol. Reprod. **19**: 761.

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Factors Affecting Reproductive Traits In Cross-bred Cows

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ABSTRACT

The data pertaining to the reproductive traits of 71 cross bred cows during the period 1982-'83 were collected and analysed to study the factors influencing the post-partum oestrus, number of Artificial insemination (AI) per conception, service period, inter-calving period and weight of calf at birth. The mean postpartum oestrus, Number of AI per conception, service period, intercalving period and weight of calf at birth were 75.37±5.21 days; 2.54±0.21; 126.08± 9.34 days; 418.83±12.92 days and 24.46± 0.65 kg. respectively. Milk yield of cows during 90 days after calving had significant influence on the number of AI per conception. Post-partum oestrus interval did not vary significantly between seasons. Number of AI per conception and service period showed significant variation between seasons. Season of calving had no significant influence on the inter calving period and birth weight of calves.

* * *

Introduction of cross bereding of indigenous cattle with exotic breeds has resulted in the production of high yieldmg animals. But to explore the full production potential of these cross bred cows it is essential that they should possess a high reproductive efficiency. Substantial economic losses result from long intercalving period and early culling of cows due to reproductive failure. The post-partum phase is the most critical period in determining the efficient reproduction in cows. It is known that certain factors like stress due to high production and variations in the climate may alter the already sensitive post-partum phase of the reproductive cycle and thereby upset the whole reproductive mechanisms. An attempt is, therefore, made to study reproductive traits of cross bred cows during the post-partum period and to find out the possible factors which would affect the reproductive behaviour during this period.

Materials and Methods

The data pertaining to 71 cross bred cows belonging to the Livestock farm attached to the Kerala Agricultural University formed the material for the study. All the cows were maintained under identical conditions of feed and management. The data with regard to the post-partum oestrous interval, number of AI per conception, inter calving period and birth weight of calves were collected. To study the effect of production on these traits the milk production during the first 90 days were collected and grouped as those producing 750 kg. and below and those producing above 750 kg. To study the effect of season, the whole year was divided into the following season as per Mathai and Raja (1976).

Season I: Summer—February, March, April and May.

TABLE 1 Showing the means and standard error of post-partum oestrus interval, number of A.I. per conception, service period, calving interval of cross bred cows and birth weight of calves during season I, II and III.

	Season I	Season II	Season III
Post-partum oestrum	66 ± 10.83 days	84.9 ± 10.52 days	75.03 ± 7.44 days
Number of A.I. per conception	2.42 ± 0.37	3.61** ± 0.36	2.06 ± 0.26
Service period	106.82 ± 18.81 dasy	171.22** ± 18.28 days	115.39 ± 12.92 days
Calving interval	398.47 ± 9.85 days	451.17** ± 9.58 days	395.72 ± 6.77 days
Birth weight of calves	23.29 ± 1.52 kg	23.56 ± 1.48 kg.	25.43 ± 1.03 kg.

** Significant at 1% level

TABLE 2 Showing the means of post-partum oestrus interval, number of A.I. per conception, service period, and calving interval in cross bred cows.

	Milk yield	
	Above 750 kg in	Below 750 kg in
	first 90 days	first 90 days
Post-partum oestrum	79.07 ± 9.28 days	72.66 ± 5.96 days
Number of A.I. per conception	3.32 ± 0.33**	2.74 ± 0.26
Service period	144.87 ± 15.8 days	114.78 ± 11.32
Calving interval	434.8 ± 16.23 days	407.15 ± 18.97 days

** Significant at 1% level

Season II: Rainy—June, July, August and September.

Season III: Winter—October, November, December and January.

The data were subjected to statistical analysis (Snedecor and Cochran, 1967).

Results and Discussion

The reproductive traits and factors influencing it are shown in Table I and II. The post-partum oestrum occurred at a mean interval of 75.37±5.21 days in cross bred cows studied. It was observed that the pot-partum oestrus interval was shorter (72.66±5·96 days) in those having production less than 750 kg during the first ninety days then those produced more than 750 kg (79.07±9.28) days. The variation was, however, not significant. This is in agreement with that of Edmondson, (1949), and Olds and Seath (1953) who reported that milk production had no influence on the timings of the first oestrus after calving. Korner (1966) in contrast, pointed out that the onset of oestrus varies within physiological limits and can be blocked by high milk performance. This was supported by Blan (1958) who observed that a high frequency of silent heat in good milkers in which overt signs of heat only appeared after milk yield declined. Mares (1959) found correlations between 90 days milk production and the calving to oestrus interval. It could also be observed that post-partum oestrus occurred in 66± 10.83 days; 84.9±10.52 days; 75.03± 7.44 days during summer, rainy and winter seasons. Analysis of data did not reveal any significant influence of seasons of calving on the onset of post-partum oestrus. However, seasonal influence on the post-partum ovarian activity has been reported by Roberts, (1971) and Hafez (1980).

The number of Al per conception was 3.32±0.33 and 2.74±0.26 in cows which produced more than 750 kg. and below 750 kg. respectively. The variation was statistically significant. This increase in number of A.1. in high yielding animals is in agreement with finding of Baier (1965) and Sell (1965). The number of A.1. per conception was 2.42±0.37 during summer, 3.61±0.36 during rainy and 2.06±0.26 during winter. Analysis of data revealed that the number of A.1. per conception was significantly higher in rainy than summer or winter season.

The mean service period of the cows was 126.08±9.34 days. Service period did not vary significantly between the high and low producers, the values being 144.87±15.8 days and 114.78±11.32 days respectively. The mean service period of cows calved during summer, rainy and winter season were 106.82± 18.81 days; 171.22±18.28 days; and 115.33±12.92 days respectively. On analysis it was found that the service period of cows calved during rainy season was significantly higher than those calved during summer and winter seasons.

The mean intercalving period of cows studied was 418.83±12.92 days. It was further revealed that the milk yield during first 90 days did not influence the intercalving period, the values being 434.8± 16.23 days and 407.15±18.97 days respectively for those which produced more than 750 kg. and less than 750 kg. of milk in first 90 days of lactation. This is consistant with the report of Carl (1967) who did not find any definite correlation between average calving interval and milk production performance. On the contrary Carman (1955) and Damn (1965) opined that high milk yield performance of cows was always accompanied by prolonged calving interval. The inter calving period in cows which had previous calving during summer, Rainy and Winter seasons was 398.47± 9.85 days; 451.17±9.58 days and 395.72± 6.77 days respectively. The calving interval of cows calved during rainy season was significantly higher than other seasons. Singh *et al.* (1958), however, found that season of calving had no influence on the future inter calving period in Hariana cows.

The mean birth weight of calves in the present study was 24.46+0.65 kg. The mean birth weight of calves born during summer, rainy and winter seasons were 23.29±1.52 kg., 23.56±1.48 kg. and 25.43±1.03 kg. respectively. The variation in the birth weight of calves due to seasons was not significant. This is in agreement with that of Mathai and Raja (1976).

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REFERENCES

Baier, W. (1965) Cit Smidt and Farries.

Blan, G. (1958) Cit Smidt and Farries.

Carman, G.M. (1955) Interrelations of milk production and breeding efficiency in dairy cows. J. Anim. Sci. **14:** 753-759.

Carl (1967) Cit Smidt and Farries.

Damm, H.J. (1965) Cit Smidt and Farries.

Edmondson, J.H. (1949) Factors affecting interval between parturition and subsequent oestrus in dairy cattle. J. Dairy Sci., **32:** 726-727.

Hafez, E.S.E. (1980) Reproduction in Farm Animals. 4th ed. Philadelphia. pp. 450.

Korner, E. (1966) Cit. Smidt and Farries.

Mathai, E. and Raja, C.K.S.V. (1976) A study on the growth rate and age at puberty of Jersy-Sindhi females under different regimes of feeding. Kerala J. Vet. Sci. **7:** 14.

Mares, S.E., Menge, A.C. and Tyler, W.J. (1959) Association of post-partum intervals with body weight loss, milk production, and butter fat percentage to 90 days following calving. 54th Annual meeting of the Amer. Dairy Sci. Assn. Urbana, Illinois.

Olds, D. and Seath, D.M. (1953) Repeatability heritability on the effect of level of milk production on the occurrence of first oestrus after calving in dairy cattle. J. Anim. Sci. **12:** 10-14.

Roberts, S.J. (1971) Veterinary obstetrics and Genital diseases. 2nd ed. Scientific book agency, Calcutta.

Sell, (1965) Cit Smidt and Farries.

Singh, R.B., Sharma, S.C. and Singh Surajit (1958) Influence of season of calving on inter calving period in Murrah Buffaloes and Hariyana Cows. Indian J. Dairy Sci. **11:** 154-160.

Smidt, D. and Farries, E. (1982) Factors influencing fertility in the post-partum cow. 1st ed. Brussels-Luxemburg. pp. 358-383.

Snedecor, G.W. and Cochran, W.G. (1967) Statistical methods. Oxford and IBH publishing Co. Calcutta. 6th ed. pp. 163-171.

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Cyclic Pattern and Occurrence Of Oestruses In Repeater Buffaloes.

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ABSTRACT

Inter-cestrual lengths of the 1121 oestrous cycles in 243 repeat breeding (R.B.) buffaloes were studied. The normal range of oestrous cycle length (18-25 days) was observed to the extent of 36·75 per cent. The short (<17 days), long (26-36 days) and double cycle lengths (37 to 48 days) were observed to the extent of 20·33, 19·89 and 23·05 per cent respectively.

Occurrence of oestrouses in repeat breeding buffaloes during different months of the year were studied. Out of 1698 oestrous periods studied, 1101 (64.8%) were exhibited during high breeding season (September-February) and 597 (35.16%) during low breeding season (March-August).

* * *

Normal reproductive efficiency is the first basis for sound and economical animal production. The need to maintain regular calvings has been advocated frequently and the cost of delayed breeding has been shown to be relatively high. The animal failing to conceive i.e. repeating adversely affects the economy of the cattle industry by loss of production, loss through the cost of maintaining non-producing animals, loss due to decreased. no. of calves born and loss due to increased depriciation cost. The reproductive cycle in the animal is chiefly governed by hormones and affected by many factors like nutritive status of the animal, seasonal influence, day light hours, temperature, age, lactational stress, transportation, systemic diseases, endocrine disturbances and infection of genital tract.

Materials and Methods

For the present study the data were collected from the Veterinary College A.I. Clinic attached to the Department of Gynaecology and Obstetrics. Records of buffaloes which had completed four or more inseminations without pregnancy were studied. In all 1121 inter-oestrus intervals from 243 (36 Heifers and 207 buffaloes) animals were analysed. These intervals were dividded into various groups as < 17 days (short cycle), 18-25 days (normal cycle), 26-36 days (long cycle) and 37-50 days (double cycle.)

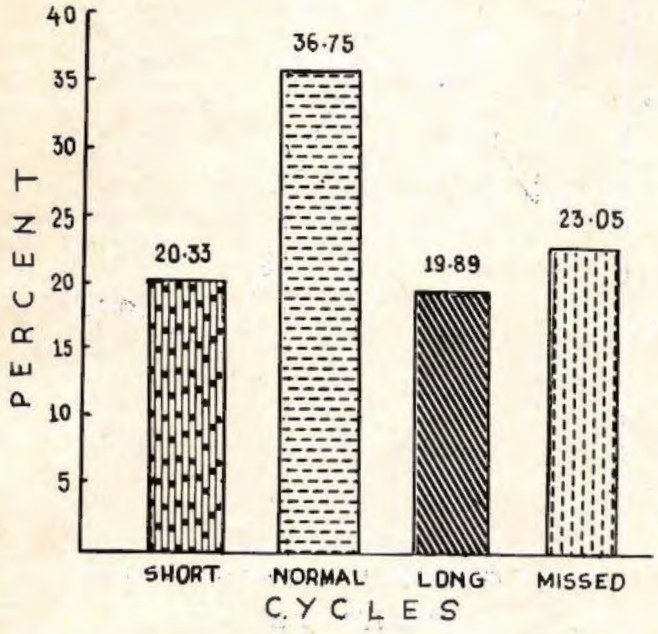
Occurrence of oestruses in R.B. Buffaloes

The breeding records of 324 R.B. buffaloes and heifers were studied and analysed to find out the frequency of repeating cycles in them during different months and during low (March to August) and high (September to February) breeding seasons of the year. A total of 1698 oestrous cycles were analysed for the above purpose.

Results and Discussion

The inter-oestrual period plays an important role in the reproductive ability of

Fig. 1 : THE PATTERN OF OESTROUS CYCLES IN REPEAT BREEDER BUFFALOES



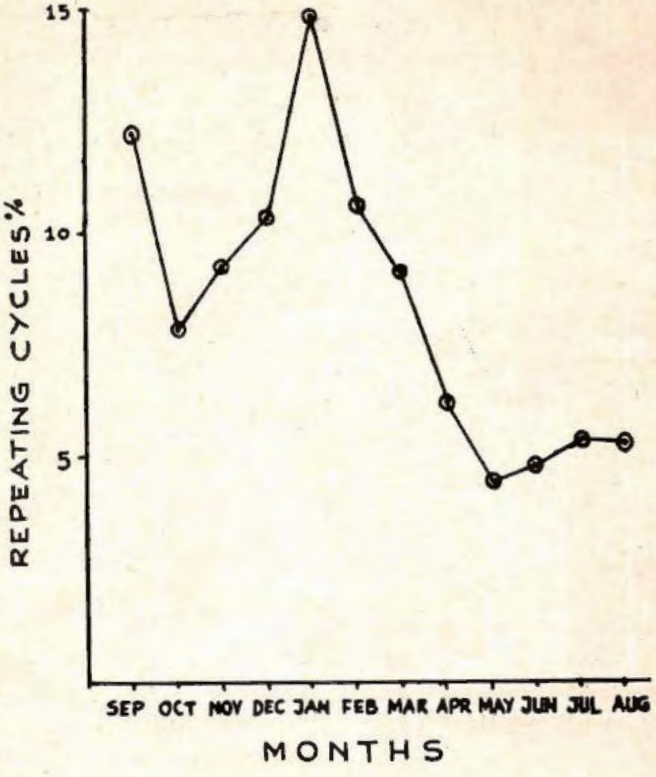
an individual female and aberrations in this pattern can lead to various subfertility conditions. In the present study on pattern of oestrous cycle in repeating buffaloes a total of 1121 oestrous cycles in 243 buffaloes were observed and studied.

The study revealed that in repeating buffaloes the number and percent of short cycle, snormal cycle, long cycle and double cycle (missed cycle) were 228 (20.33%), 412 (36.75%), 223 (19.89%) and 258 (23.50%) respectively. The trend of repeating cycles has been presented in Figure 1.

Hafez (1954) reported that symptoms of heat in buffaloes were of less intensity. As a result, the interval between two successive inseminations increases. Luktuke (1976) reported on the aberrant patterns of oestrus and oestrous cycles occuring in buffaloes to be due to weak oestrus, silent oestrus, early ovulation, delayed ovaluation, anovulatory oestrus, short and long oestrus and persistant oestrus. His study revealed that long oestrous cycles (<26 days) were of frequent occurence in repeating buffaloes.

Rao *et al.* (1973) reported that 40

Fig:2: REPEAT BREEDING IN BUFFALOES.



percent of the cycles in Surti buffaloes fell within the range of 15 to 29 days. Derashri (1982) reported that 45.07 percent of oestrous cycles occurred within normal length (18 to 24 days) and 54.93 percent cycles were with abnormal length. Under the present study the normal cyclicity was 36.75 percent. The normal pattern of cyclicity should be at least 70 percent in regular breeding animals (Lamond, 1968). It was apparent that in repeat breeding buffaloes the normal pattern of oestrous cycle length was markedly affected.

Ginther (1968) reported that exposure of the endometrium early in the oestrous cycle to experimental metritis or foreign substances resulted in shortened oestrous cycles, whereas, exposure late in the cycle to metritis resulted in prolonged cycles.

In repeat breeder buffaloes in present study, the percentage of short and long cycles were to the extent of 20.33 and 19.89 percent, respectively. In assessing the repeat breeder syndromes the effect of death of conceptus on cycle length is an important consideration. It has been reported that embryonal mortality occur-

red within 16-35 days in a considerably large percentage of R.B. animals (Hansel, 1959, Boyd *et al.* 1969) and this was usally associated with longer cycles.

Occurrence of oestrus in Repeat Breeders:

Under the present study a total of 1698 oestrous periods were studied in 324 repeat breeder buffaloes and heifers. An average of 5.24 (3.0 to 12.0) insemina-tions were performed without conception. Out of 1698 oestrous periods 1101 (64.84%) were exhibited during breeding season (September to February) and 597 (35.16% during low breeding season (March to August). The details have been presented in figure 2.

Narsimha Rao and Sambashiva Rao (1970) reported that oestrus in village herds of Indian water buffaloes has a definite breeding period. Seasonality of breeding in the Indian breeds of bufialoes has been reported by Roy *et al.* (1972) and Abhi *et al.* (1973).

It can be seen that though the animals under study were repeat breeder buffaloes the ratio of frequencies of evinced oestrous periods per animal in breeding and low breeding seasons was differed significantly (3.39 vs 1.84). It is evident that the problem of repeat breeding is being faced in the field more during the breeding season when animals evince more number of oestrous period.

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REFERENCES

Abhi, H.L., Nagpal, N.P. and Sharma T.L. (1973). Indian J. Dairy Sci., **26**: 107-109.
Boyd, H., Bacsich, P., Young, A. and McCracken, J.A. (1969). Brit. Vet. J. **125**: 87-89.
Derashri, H.J. (1982). M.V.Sc. thesis, Gujarat Veterinary College, G.A.U., Anand.
Ginther, O.J. (1968). J. Amer. Vet. Med. Assoc. **153**: 1665: 1665-1671.
Hafez, E.S.E. (1954). Ferti & Steril, **5**: 482-491.
Hansel, W. (1959). Ferti & Steril, **10**: 502.
Luktuke, S.N. (1976). Annual Scientific Report. Dept. of Animal Reprod. I.V.R.I., Izatnagar.
Narshinh Rao and Sambashiva Rao, C. (1970). Indian Vet. J., **47**: 742-74.
Rao, B.R., Patel U.G., and Tamhan S.S. (1973). Indian Vet. J. **50**: 257-264.
Roy, D.J., Bhattacharya A.R. and Luktuke, S.N. (1972). Indian Vet. J., **50**: 257-264.
Roy, D.J., Bhattacharya, A.R. and Luktuke, S.N. (1972). Indian Vet. J., **49**: 54-68.

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Studies On Blood calcium And Phosphorus In Anestrus Crossbred Cows

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ABSTRACT

The study was conducted on 80 crossbred cows to observe the blood calcium and phosphorus level in anestrus cows. The mean calcium value (10.18 mg per cent) on the day of heat was significantly higher than the value (9.97 mg per cent) when the animals were in anestrus. The level of inorganic phosphorus at estrus averaged 7.79 mg percent with average of 3.8 to 12.19 mg per cent, while during anestrus, the value averaged 6.84 mg per cent with range of 3.2 to 11.88 mg per cent. The difference in the values was significant ($P<0.05$).

* * * *

There is a definite role of calcium and phosphorus in female reproduction. Lack of minerals especially calcium and phosphorus upset the proper functioning of reproductive organs. Moddie (1965) described the role of calcium in sensitizing the tubular genitalia for action of hormones.

Hence the study was planned to investigate the biochemical constituents in anestrus crossbred cows.

Materials and Methods

The study was done on crossbred cows belonging to Military Dairy Farm, Jabalpur. The experimental animals comprised of 80 anestrus crossbred cows. Animals were divided into following groups:-

Group	Number of cows in the group	Type of groups	Name of groups
I	20	Control	Anestrus
IIa	10	Treatment	Fertivet
IIb	10	Treatment	Fertivet+ Copper sulphate
III	20	Treatment	Secrodyl
IV	20	Treatment	Lugol's iodine with utero-ovarian massage.

Anestrus group Control: No treatment was given to the cows of his group. Blood samples were collected on the day of gynaeco-clinical examination. Each animals of this group was observed for signs of heat up to 21 days. Blood samples were collected from the cows that came in heat within the above period on the day of induction of heat and also from the cows that failed to come in heat within the above period on day 21 of gynaeco-clinical examination.

Treatment group: Blood samples from all the cows in these groups were collected just prior to treatment and after gynaeco-clinical examination. Each animal was

observed for signs of heat upto 21 days. If they came in heat on any day post-treatment, their blood was collected on the day of induced heat within the above period. The blood samples of the animals which failed to come in heat upto 21 days were obtained on twenty first day of gynaeco-clinical examination.

Estrus group: Blood samples from the cows of this group were obtained on the day of induction of heat after gynaeco-clinical examination. The concentration of different blood chemical constituents was compared with the corresponding values recorded during anestrurs.

Results and Discussion

In the present study the serum calcium value averaged 9.98 mg per cent with the range of 7.7 to 13.0 mg per cent in anestrus cows. The corresponding value on day of induced heat ranged from 8.2 to 12.3 mg per cent with an average value of 10.18 mg per cent (Table 1).

TABLE 1 Serum calcium concentration (mg%) in different group of crossbred cows.

Group	Pre-treatment (mg%)		Post-treatment (mg%)	
	Mean±S.E.	Range	Mean±S.E.	Range
I	9.85±0.29	7.7—13.0	9.97±0.33	7.2—13.2
IIa	9.98±0.34	8.8—11.8	10.11±0.39	8.2—11.8
IIb	9.82±0.36	8.5—11.6	10.05±0.39	8.4—11.9
III	10.04±0.20	8.5—11.2	10.29±0.23	8.3—12.7
IV	9.76±0.27	7.8—12.2	9.84±0.27	7.5—12.3
0	9.97±0.17	7.9—12.0	10.18±0.18	8.2—12.3

Present findings almost resemble with Setty and Razdan (1966) who reported the level as 9.33 mg per cent in Tharparkar and 9.59 mg per cent in Sahiwal. Average value of 10.75 mg per cent reported by Mithuji *et al.* (1966) and 10.42 to 10.75 mg per cent reported by Acharya *et al* (1968), are nearer to the present findings. Crookshank and Frank (1955) reported higher value of serum calcium (11.08 mg per cent with range of 9.4 to 12.4 mg per cent) in Hereford cows. Bhosrekar *et al* (1967) and Patel *et al* (1966) opined that season influenced the blood level of calcium. They recorded highest value (10.40 mg per cent) in summer and lowest (9.3 mg per cent) in rainy season. The average value (10.18 mg per cent) recorded during the present study is in agreement with the reports of these workers. The variation in the blood calcium level in the present investigation may be attributed to difference in age of the experimental animals, as Payne and Leech (1968) have reported that calcium level tended of decrease with advancement in age of cows.

There was no significant difference in the pre and post treatment calcium value in all the groups except the estrus group. In this, mean calcium value (10.18 mg per cent) on day of heat was significantly higher than the value (9.97 mg per cent) when the animals were in anestrus. This is in agreement with the observations of Mokashi *et al* (1974) and Samad *et al* (1980) who also reported higher values of serum calcium on day of heat in cows as 8.63 and 9.6 mg per cent as compared to 8.44 mg per cent and 9.2 mg per cent in anestrus cows.

Inorganic Phosphorus:

The level of inorganic phosphorus at estrus averaged 7.79 mg per cent with average of 3.8 to 12.19 mg per cent, while during anestrus, the value averaged 6.84 mg per cent with range of 3.2 to 11.88 mg per cent (Table 2).

The difference in the values was significant ($P<0.05$). The normal value of serum inorganic phosphorus was 5.56 per cent (Crookshank and Frank 1955) and 5 mg per cent (Payne and Leech

TABLE 2 Serum inorganic phosphorus concentration (mg%) in different groups of crossbred cows

Group	Pre-treatment (mg%)		Post treatment (mg%)	
	Mean±S.E.	Range	Mean±S.E.	Range
I	6.58±0.34	4.12—9.82	6.44±0.36	4.21—9.98
IIa	7.09±0.53	4.42—9.32	6.95±0.54	4.17—9.42
IIb	7.33±0.63	4.32—10.82	7.59±0.61	4.82—11.13
III	6.53±0.55	3.2—9.72	7.04±0.49	3.27—10.7
IV	6.66±0.56	3.47—11.88	6.89±0.54	3.87—12.12
0	7.48±0.35	3.98—11.88	7.79±0.34	3.8—12.19

1964). In the present study the blood level of phosphorus was more at the time of estrus and thus finding is in agreement with the report of Mokashi *et al* (1974) Dindorkar and Kohli (1979) and Samad *et al* (1980). The low level of phosphorus may be one of the cause/s of anestrus in the present study.

The role of phosphorus in regulation of estrus rhythm has been established. Acharya (1960) observed that lack of phosphorus upsets the proper function of reproductive organs. Becze (1964) reported that deficiency of dietary phosphorus as a result of exhaustion of reserves during lactation may have caused anestrus. The most prevalent mineral deficiency affecting reproduction appeared to be the lack of phosphorus (Salisbury and VanDemark 1964). Mahadevan (1963) observed delay in sexual maturity and irregularity in estrus due to direct or indirect deficiency of phosphorus. Maynard and Loosly (1969) stated that reproductive problems are common in phosphorus deficient pastures. It is indicated (Table 2) that the average pre-treatment phosphorus level in group III (secrodyl) increased from 6.53 to 7.04 mg per cent and in roup IV (Lugols iodine) from 6.66 mg per cent to 6.89 mg per cent. These differences were statistically significant at 1 and 5 per cent level respectively. Lack of phosphorus as a result of exhaustation of reserves during lactation and pregnancy may be responsible for anestrus (Becze 1964). Sane (1977) reported that high calcium concentration suppresses phosphorus and Manganese utilization, and in phosphorus deficient ration, the symptoms of estrus were suppressed to such an extent that even with a vasectomised bull the heat detection was difficult.

Patel *et al* (1966) and Bhosrekar *et al* (1967) concluded that slight discrepancy in values of serum inorganic phosphorus level at estrus and in anestrus animals may be due to differences in environmental conditions prevailing during the study.

In the present investigation, phosphorus to calcium ratio was higher (1:1.33) in anestrus cows as compared to the value (1:1.31) on the day of induced estrus. Decrease in the ratio was due to increase in the level of phosphorus in cows on the day of induced heat (Table 3).

TABLE 3 Serum phosphorus and Calcium ratio in different groups of crossbred cows.

Group	Pre-treatment (P:Ca)	Post-treatment (P:Ca)
I	1:1.50	1:1.55
IIa	1:1.41	1:1.45
IIb	1:1.34	1:1.32
III	1:1.54	1:1.47
IV	1:1.47	1:1.43
0	1:1.33	1:1.31

Various workers have reported that there is an increase in both calcium and phosphorus level at the time of heat as compared to anestrus condition. Higher values of calcium at the time of heat were reported by Mokashi *et al* (1974) and Samad *et al* (1980).

Similarly higher phosphorus values at the time of heat were reported by Dindorkar and Kohli (1979). According to these reports when values of both calcium and phosphorus are higher at estrus it should not affect the calcium and phosphorus ratio because both are increasing at estrus. Little John and Lewis (1960) could not show any significant relationship between calcium-phosphorus ratio of diet and fertility in dairy heifers. They also concluded that high intake of phosphorus depressed serum calcium even when the intake of calcium was high. Lactation makes heavy demand for calcium and phosphorus and is thus important for animals. An excess of calcium supplement in diet may be responsible for deficiency of phosphorus (Ford, 1965).

REFERENCES

Acharya, A.K., Gupta, S. and Bhattacharya, S. (1968) Studies on serum calcium, inorganic phosphorus and Magnesium concentration of blood during pregnancy in different breeds of Indian Dairy Cattle. *Indian J. Dairy Sci.* **21**: 204-207.

Acharya, R.M. (1960) A note on infertility of cows. *Indian* Bet. *J.* **37**: 417-422.

Becze, J. (1964) Nature of sterility of nutritional organin in cattle in Hungary. Wein tierarztt Mschr. **51**: 289-300.

Bhosrekar, M.R., Daniel, S.J. and Mullick, D.N. (1967) A study of pulse and respiration rates, rectal temperature and blood serum composition of lactating dairy cows. *Indian J. Dairy Sci.* **20**: 49-52.

Crookshank, H.R. and Frank, H.S. (1955) Serum values in wheat pasture poisoning cases. *J. Anim. Sci.* **14**: 964-969.

Dindorkar, C.V. and Kohli, I.S. (1979) Variations in blood serum, inorganic phosphorus, calcium, magnesium values of cattle having regular estrus cycles and those in anestrum. *Indian J. Anim. Health.* **18**: 25-27.

Ford, C.M. (1965) Infertility in farm animals in Britain. *Brit. Vet. J.* **121**: 42-50.

Little John, A.I. and Lewis, G. (1960) Experimental Studies of the relationship between calcium phosphorus ratio of the diet and fertility in heifers — A preliminary report. *Vet. Rec.* **72**: 1137.

Mahadevan, V. (1963) Feed as a factor of fertility. *Indian Livestock.* **1**: 6-8.

Maynord, L.A. and Boosli, J.K. (1969) *Animal Nutrition* 6th Ed. Tata McGraw-Hill Publishing Company Ltd. Bombay.

Mithuji, G.F., Shukla, P.C. and Patel, B.M. (1966) Haematological studies in Kankrej Cattle. *Indian Vet. J.* **43**: 605-612.

Mokashi, B.G., Deshpande, B.R. and Sane, C.R. (1974) Study of post-partum anoestrus condition and effectiveness thereon of the increased energy intake. *Indian. Vet. J.* **51**: 671-675.

Moddie, E.W. (1965) Hypocalcaemia and Hypomagnesaemia. *Brit. Vet. J. 121*: 338-349.

Patel, B.M., Menon, G.N. and Mistry, V.V. (1966) Inorganic phosphorus, calcium and magnesium levels in the serum of Gir Cattle. *Indian Vet. J.* **43**: 795-800.

Payne, J.M. and Leech, F.B. (1964) Factors affecting plasma calcium and inorganic phosphorus concentration in the cow with particular reference to pregnancy, lactation andage. *Brit. Vet. J.* **120**: 385-387.

Salisbury, G.W. and VanDemark, N.L. (1961) *Physiology of production and artificial insemination in cattle.* W.H. Freeman and Co., London.

Samad, A., Ali, K.M. and Rahman, A (1980) Studies on certain blood constituents of anoestrus cattle. *Indian Vet. J.* **57**: 135-138.

Sane, C.R. (1977) Anestrus condition in cows. First All India Symposium on Animal Reproduction P.A.U. Ludhiana

Setty, S.V.S. and Razdan, M.N. (1966) Studies on the chemical composition of blood in Dairy Cattle I. *Indian J. Dairy Sci. 19*: 55-59.

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Bacteriological Studies Of Bovine Semen

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ABSTRACT

The present investigation reports bacteriological studies of semen collected from 45 bulls belonging to three A. I. Centres of Gujarat State during 1982-83.

The semen quality was found fairly good irrespective of bacterial load. Bacterial contamination of semen was higher in summer season followed by monsoon and winter seasons. The bacterial load of diluted semen was in proportion to that of neat semen except in case where additional contamination had taken place. The beneficial effect of dilution and antibiotics as well as freezing was observed on bacterial load. The bulls above six years of age have shown higher bacterial load of neat semen than the younger bulls. The managemental practices followed at respective Centre have rightly reflected on bacterial load of semen. Isolation of bacterial organisms from 113 neat semen samples revealed no significant difference in frequency of occurrence of gram negative (50.81%) and gram positive (49.19%) isolates. Among all the isolates, Bacillus spp. was highest in predominance.

* * *

The collection of semen is generally done out doors and laboratory conditions are not ideal everywhere. It is, therefore, difficult to collect semen completely free from microorganisms even under strict hygenic conditions and some amount of microbial contamination is bound to be expected. Contamination of fresh and preserved semen with microorganisms poses a great threat to the successful Artificial Insemination programme. Unfortunately, in our country, little emphasis is given to microbial load of semen and very limited work has been done on this aspect (Mahmoud, 1953; Singh, 1963; Dholakia *et al.*, 1970; Reddy *et al.*, 1971; Khandekar, 1973 and Naidu *et al.*, 1982). In view of this, the present investigation was undertaken to study bacteriological aspect of bovine semen to find out effect of species, breed, season, managemental practices, age of bull, dilution, addition of antibiotics and freezing on bacterial load of semen and to correlate bacterial load with other laboratory tests used to assess semen quality. It was also aimed to determine the types of bacterial organisms present in neat semen.

Materials and Methods

Materials for present study were collected from 45 bulls stationed at three A. I. Centres belonging to Department of Animal Husbandry, Gujarat State during Summer (March-June), Monsoon (July-October) and Winter (November-February) seasons of the year 1982-83. A questionaire was prepared to get information regarding managemental practices followed at respective centre.

TABLE 1 Spermogramme showing seasonwise mean values of semen characteristics on different centres.

Sr. No.	Parameters	Regional A.I. Centre, Rajkot			Central Semen Collection Station, Mehsana			A.I. Centre, Godhra		
		Summer Season	Monsoon Season	Winter Season	Summer Season	Monsoon Season	Winter Season	Summer Season	Monsoon Season	Winter Season
1	Semen volume in ml.	8.45 (5-16)	7.56 (5-13)	7.00 (3.5-11)	8.48 (5-12)	7.12 (3.5-11.5)	7.33 (3-11.5)	4.00 (3-5)	4.88 (3-7.5)	4.63 (3.5-6)
2	Mass activity	+++(+)	++++	+++(+)	++++	++++	+++(+)	++++	++++	++++
3	Density	DD(D)	DD(D)	DD(D)	DDD	NT	DDDD	NT	NT	NT
4	Motility in per cent	73.75 (70-80)	73.55 (70-80)	70.71 (70-80)	76.00 (70-08)	73.82 (70-80)	72.78 (65-80)	71.25 (65-75)	73.57 (65-80)	72.50 (70-80)
5	pH	6.45 (6.2-6.8)	6.33 (6.1-6.5)	6.56 (6.4-6.9)	6.81 (6.6-6.9)	6.81 (6.7-6.9)	6.79 (6.6-6.9)	6.53 (6.3-6.7)	6.55 (6.4-6.7)	6.48 (6.3-6.6)
6	Live sperm %	81.58 (74-92)	80.24 (75-83)	79.00 (71-84)	87.07 (75-92)	90.35 (85-95)	92.00 (85-95)	82.25 (75-85)	86.00 (80-90)	86.75 (85-89)
7	Abnormal sperm %	NT	NT	NT	7.73 (4-12)	6.88 (4-11)	6.09 (3-11)	13.56 (10-21)	11.25 (6-16)	12.25 (8-16)
8	Bacterial count per ml of neat semen	9,766 (3,850-17,000)	4,122 (1,760-9,600)	3,405 (520-8,500)	5,373 (1,330-9,700)	3,573 (2,150-6,400)	2,456 (1,280-4,000)	14,375 (10,300-21,500)	11,725 (9,300-16,500)	8,717 (3,600-6,200)

NT = Not tested. Parenthesis indicate the range.

TABLE 2 Seasonwise average bacterial load from neat, diluted and frozen semen of different breeds.

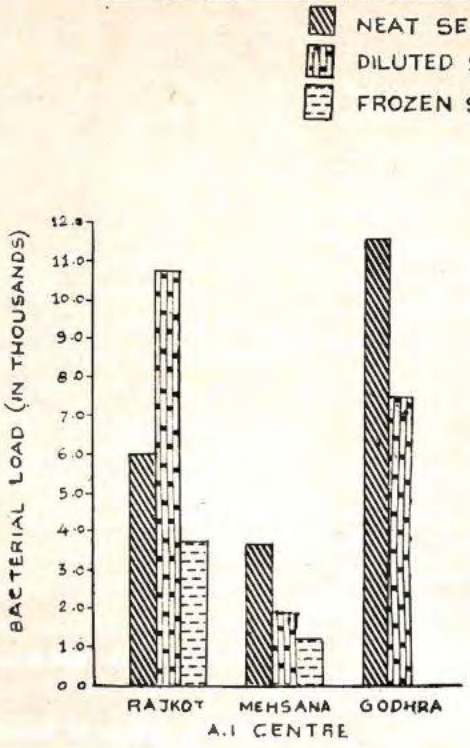
Breed	No. of bulls	Summer season			Monsoon season			Winter season		
		Bacterial load per ml of			Bacterial load per ml of			Bacterial load per ml of		
		Neat semen	Diluted semen	Frozen semen	Neat semen	Diluted semen	Frozen semen	Neat semen	Diluted semen	Frozen semen
Gir*	7	11,471 (7200—15,500)	35,757 (18,00—56,000)	14,500 (13,900—15,100)	4,860 (3,00—8,100)	2,091 (905—3,950)	1,073 (460—1,550)	4,980 (3,200-6,000)	2,505 (1,210-3,200)	1,183 (340-1,730)
Jaffarabadi*	6	11,920 (5,600—17,000)	27,480 (9,100—(39,000)	14,400 (6,100—20,100)	6,328 (2,860—9,600)	1,753 (1,380—1,980)	1,320 (910—1,520)	5,325 (3,700-8,500)	2,485 (1,980-2.920)	793 (460-1,410)
Jersey*	7	6,940 (3,850—9,000)	15,740 (8,000—20,600)	10,500 (9,300—11,700)	2,818 (2,530—3,150)	1,575 (1,300—2,210)	955 (640—1,270)	1,990 (1,110-4,100)	1,225 (510-3,200)	591 (280-1,530)
Holstein Friesian*	2	6,300 (5,900-6,700)	13,600 (11,300-15,900)	4,600 (4,200-5,000)	1,800 (1,500-2,100)	1,100 (800-1,400)	286 (270-302)	630 (520-740)	370 (320-420)	98 —
Jersey* × Gir	1	4,600	14,200	7,800	1,760	1,110	660	750	290	115
Murrah**	18	5,373 (1,330-9,700)	2,835 (860-6,500)	1,687 (360-5,500)	3,575 (2,150-6,400)	1,701 (790-2,720)	979 (240-1,730)	2,456 (1,280-	1,358 (660-	286 (260-
Surti+	4	14,375 (10,300-(21,500)	10,700 (7,200-16,200)	NT	11,725 (9,300-16,500)	8,475 (7,300-12,300)	NT	4,000) 4,675 (3,600-6,200)	2,610) 3,265 (2,300-4,700)	1,220) NT

NT = Not tested * Regional A.I. Centre, Rajkot

** Central Semen Collection Station, Mehsana + A.I. Centre, Godhra

Parenthesis indicate range of the bacterial load.

Fig. 1 ANNUAL AVERAGE OF BACTERIAL LOAD FROM NEAT, DILUTED AND FROZEN SEMEN AT DIFFERENT A.I CENTRES.



Approximately, 1.5 ml of each neat semen and diluted semen (with antibiotics in usual proportion) were collected in sterilized vials and brought to laboratory on ice, while for frozen semen, three straws each with 0.5 ml of frozen semen preserved in liquid nitrogen were brought to the laboratory. Routine laboratory tests viz. pH, density, mass activity, motility, live count and abnormal spermatozoa were carried out on the spot.

The bacterial count of samples was estimated by Standard Plate Count (SPC) method (Cruickshank *et al.*, 1974). Primary isolation of organisms was done on blood agar. On the basis of cultural characters and morphology of stained smears, the organisms were grouped as gram positive and gram negative. Gram positive isolates were further identified as per Bergey's manual of determinative bacteriology (Buchanan and Gibbsons, 1974) and Cowan and Steel (1970), while gram negative organisms were identified using strips as well as criteria tables especially devised by the Department of Bacteriology and Hygiene at Royal Veterinary College, Copenhagen.

Results and Discussion

A. *Semen quality:*

The seasonwise mean values of semen characteristics on each centre are presented in table-1.

In all the cases, semen quality was found fairly good, irrespective of bacterial load, however in some cases, results of fertility were not satisfactory viz. nt Regional A. I. Centre, Rajkot, *Pseudomonas aeruginosa* organisms were constantly isolated from neat semen of two bulls and results of fertility were poor. This may possible due to spermogramme carried out immediately after collection of semen and organisms might not have found sufficient time to cause detrimental effect on sperm. This might also be due to resistance of these organisms to commonly used antibiotics because of their festidious nature.

B. *Bacterial load:*

The annual average of bacterial load from neat, diluted and frozen semen at all the three centres is shown in fig. 1, while seasonwise average bacterial load from neat, diluted and frozen semen is presented in table-2.

Bacterial contamination was higher in summer season followed by monsoon and winter seasons. The results were in accordance with that of Dholakia *et al.* (1970) where they found more contamination in summer season than in winter season.

Three A. I. Centres included in the present study have significant differences in the environmental conditions and the managemental practices. At all the Centres, environment provided for bulls and precautions taken during collection, processing and preservation of semen have rightly reflected on bacterial load of semen.

The bacterial count of diluted semen was in proportion to that of neat semen except in case where additional contamination had taken place. This particularly, is true for Regional A. I. Centre, Rajkot during summer season where count of diluted semen was found even higher than that of neat semen. This might be due to either using contaminated diluter and/or resistance of the organisms to routinely used antibiotics. Lower count of diluted semen encountered at Central Semen Collection Station, Mehsana may probably because of addition of gentamycin along with Strepto-penicillin in diluter.

In the present study, bacterial load of frozen semen which varied from 98 to 14,500 organisms per ml, is low as compared to that reported by Khandekar (1973) and Pospelov *et al.* (1973) where they encountered average bacterial count of frozen semen 30,000 and 75,000 per ml respectively. Count of frozen semen was lower than that of diluted semen in each case indicating effect of freezing on total bacterial load.

Breedwise annual average of bacterial load from neat semen is shown in table-3.

TABLE 3: Effect of breed on bacterial load of neat semen.

Breed	Number of bulls	Average Bacterial load per ml of neat semen
Gir	6	7275
Jaffarabadi	4	8504
Jersey	6	4204
Murrah	18	3584
Surti	4	10258

Bacterial load from neat semen was highest in Surti breed and lowest in Murrah breed which were stationed at different centres. Thus the count was highly influenced by managemental practices followed at respective centre rather than the type of breeds. However, within the centre, for example, at Regional

A. I. Centre, Rajkot bacterial count of neat semen was higher in buffalo bulls than in cow bulls. The results were controversial at this point with that of Reddy *et al.* (1971) and Krishnamurthi, *et al.* (1981) where they reported that bacterial count of neat semen and preputial washings varied according to the type of sheath which is tucked up in buffalo bulls and pendulous in cow bulls.

Agewise bacterial load of neat semen is presented in table-4.

TABLE 4 Effect of age of bulls on bacterial load of neat semen.

Age of bulls	No. of bulls	No. of samples	Average bacterial load per ml of neat semen
Young (≤6 yrs)	14	35	3235
Old (>6 yrs)	26	73	6474

It can be seen from the table that the bulls above six years of age have shown two times more bacterial load of neat semen than the younger bulls. The findings of Dholakia *et al.* (1970) and Khandekar (1973) also support the present conclusion. Reddy *et al.* (1977) also reported the lower bacterial count of neat semen in younger bulls than in older bulls.

C. *Bacteriology of neat semen:*

Out of 113 neat semen samples subjected to bacteriological examination, 86 samples yielded single type of organisms, 16 samples revealed two types of organisms and two samples resulted in isolation of three types of organisms. The rest nine samples were found bacteriologically negative. The frequency of occurrence of different isolates is shown in table-5.

Table 5 Isolation and Identification of organisms from neat semen.

Sr. No.	Organisms	No. isolated	Per cent
1	Gram negative bacilli		
	Pseudomonas aeruginosa	18	14.52
	Escherichia coli	6	4.84
	Proteus tulgaris	5	4.03
	Proteus morgani	1	0.81
	Enterobacter cloacase	5	4.03
	Enterobacter liquefaciens	3	2.42
	Hafnia alvei	1	0.81
	Alcaligenes faecalis	2	1.61
	Alcaligenes bronchosepticus	2	1.61
	Aeromonas liquefaciens	2	1.61
	Klebsiella aerogenes	2	1.61
	Acinetobacter anitratus	1	0.81
	Acinetobacter lwoffi	1	0.81
	Citrobacter Ballerup Bethesda	1	0.81
	Citrobacter freundii	1	0.81
	Flatobacterium meningosepticum	1	0.81
	Unidentified gram negative bacilli	9	7.26
2	Gram positive cocci		
	Staphylococcus aureus	4	3.23
	Staphylococcus epidermidis	15	12.10
	Micrococcus spp.	6	4.84
	Streptococcus faecalis	3	2.42
	Streptococcus agalactiae	1	0.81
	Streptococcus dysgaladctiae	1	0.81
	Streptococcus durans	1	0.81
	Streptococcus lactis	1	0.81
	Streptococcus hominis	1	0.81
	Streptococcus mitis	1	0.81
	Streptococcus uberis	1	0.81
3	Gram positive bacilli without spore		
	Corynebacterium equi	2	1.61
	Corynebacterium xerosis	1	0.81
4	Gram positive bacilli with spore		
	Bacillus coagulans	9	7.26
	Bacillus polymixa	7	5.65
	Bacillus macerans	4	3.23
	Bacillus subtilis	1	0.81
	Bacillus circulans	1	0.81
	Bacillus megaterium	1	0.81

It is evident from the table that there was no significant difference in the frequency of occurrence of gram negative (50.81%) and gram positive (49.19%) organisms. *Bacillus* spp., has shown highest percentage followed by *Pseudomonas*

aeruginosa, Staph. epideridis, Streptococci and other organisms. Marinov *et al.* (1966) Reddy *et al.* (1971), Khandekar (1973), Brown *et al.* (1974) and Naidu *et al.* (1981) have also reported occurrence of similar organisms from bovine semen except Citrobacter and Aeromonas which have only been reported in the present investigation. Edmondson *et al.* (1949), True-Blood *et al.* (1956), Sorel (1961) and Naidu *et al.* (1982) have reported *Bacillus* spp. as predominant type of organism from semen. These findings also support the present investigation.

Isolation of *Pseudomonas* spp., *E. coli, Staph. aureus, Klebsiella* spp. and non haemolytic *Streptococci* in the study is significant as these organisms have been reported to be abnormal microflora of semen by Schwerdtner (1961), Roberts (1971), Sorel (1961) and Singleton and Simmons (1969).

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REFERENCES

Brown, V.G., Schollum, L.M. and Jarvis, B.D.W. (1974). Newzealand J. Agr. Res. **17:** 435. (Vet. Bull. **45:** 2225)

Buchanan, R.E. and Gibbsons, N.E. (1974). Bergey's Mannual of Determinative Bacteriology. 8th Ed. The W Williams and Wilkins Company, Baltimore.

Cowan, S.T. and Steel, K.J. (1970). Mannual for the identification of Medical Bacteria. 1st Ed. Cambridge University Press, London.

Cruickshank, R., Duguid, J.P., Marmoin, B.P. and Swain, R.H.A. (1975). Medical Microbiology. 12th Ed. Vol. II. Churchil Livingstone, Edinburgh.

Dholakia, P.M., Bhavsar, B.K., Kodagali, S.B. and Jhala, P.M. (1970). Guj. Vet. **4:** 19-23.

Edmondson, J.E., Tallman, K.A. and Herman. H.A. (1949). Bull. Miss. Agric. Exp. Sta. **444:** 12 (Vet. Bull. **20:** 2711).

Khandekar, P.C. (1973). M.V.Sc. thesis. Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

Krishnamurthi, P.S., Pattabiraman, S.R. and Venkataswami, V. (1981). Cherion. **6:** 291

Mahmoud, I.N. (1953). Indian J. Dairy Sci. **6:** 197-200.

Marinov, P. (1970). Vet. Med. Nauki, Sofi. **7:** 81-87. (Vet. Bull. **40:** 4260)

Naidu, **X**.S., Rao, A.R. and Rao, V.P. (1982). Indian Vet J. **59:** 91-95.

Pospelov, A.I., Artemev, V.I. and Safershaev, M.A. (1973). Sbornia Robot, Leningradskii Veterinari Institute. **34:** 109-111. (Vet. Bull. **45:** 6046).

Reddy, B.J.C., Krishnamurthi, P.S. and Venkataswami, V. (1971). Indian Vet. J. 48: 722-727.

Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. IInd Ed. Scientific Book Agency, Calcutta.

Schwerdtner, H. (1961). Zuchthyg: Fortpfistorung. U. Besamug. **5:** 260-267. (Vet. Bull **32:** 670).

Singh, G. (1962). Agra. Univ. J. Res. (Science). *11:* 321. Cited by Day and Chawdhari (1965).

Singleton, E.F. and Simmons, G.C. (1969). J. agric. anim. sci. **26:** 1-7. (Vet. Bull. **40:**396).

Sorel, P. (1961). Bull. Acad. Vet. Fr. **34:** 295-300. (Vet. Bull. **32:** 1063).

True-Blood, Morrison, S.M. and Hill, H.J. (1956). J. Dairy Sci. **39:** 1481.

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Studies On The Pathological Conditions Of Genital Organs Of Cows In Assam.

III. Abnormalities in Uterus.

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SUMMARY

Four hundred and thirty genitalia of cows have been examined from the different slaughter houses of Assam of which 116 showed the abnormalities in the uterus. The incidence was 26.0 per cent of the animal examined. Anatomical deformities were recorded in 3 animals and endometritis in 87 animals being the highest incidence. The gross and microscopic pathology of these conditions were recorded and discussed.

* * *

Abnormalities in the genital tract have been documented by Sharma *et al.* (1968); Dwivedi (1968) and Nair and Raja (1974). The present communication is to place on the record abnormalities of uterus that are recorded in the slaughtered cows of Assam in a period of 15 months survey.

Materials and Methods

Four hundred and thirty genitalia of cows have been collected from the different slaughter houses of Assam and out of it 116 showed gross abnormalities. Tissues from these genitalia were collected in formol saline solution. Sections were cut in 4 to 6 micron thickness and stained with haematoxylin and eosin. MacCullum Good pasture stain, acid fast stain and other special procedures were employed whenever found necessary.

TABLE: The incidence of abnormalities recorded in uterus.

Condition observed	Number of organs affected	Percentage
Anatomical Defects		
Uterus unicornis	1	0.23
Short uterine horn	2	0.46
Miscellaneous Morbid changes:		
Sub-serous cyst	2	0.46
Pyometra	4	0.93
Uterine abscess	1	0.23
Endometritis	87	20.23
Mucometra	5	1.16
Melanosis	3	0.69
Haemosiderosis	5	1.16
Lymphoid hyperplasia on the serosal surface	1	0.23
*Leiomyoma	1	0.23

* Described elsewhere.

Results and Discussion

The results of the study on the abnormalities of uterus are being presented in the table. These abnormalities were grouped into anatomical defects and miscellaneous morbid changes resulting from pathological reactions. The uterus was affected in 26.9 per cent of animal examined.

Altogether, 3 animals showed anatomical abnormalities out of which in one case the left horn was completely absent, but the fallopian tubes were normal along with the ovaries. Short uterine horn was recorded in 2 animals and in both the cases the other parts of the genitalia were well developed. Microscopical examination of it however, did not reveal any appreciable change. Short uterine horn and uterine unicornis were recorded by several other workers (Perkin *et al.* 1954; Nongbri, 1977). Roberts (1971) suggested that these were of heriditary origin, although, some might be congenital. Two animals showed the presence of subserous cyst where a large numbers of cysts were situated on the dorsal surface measuring about 0.5-2.0 cm (Fig. 1) in one case, while in the other a single cyst was situated at the external bifurcation of the uterine horn. Microscopical study revealed that the cysts were made up of connective tissue lined by flattened epithelial cells internally. The incidence of subserous cysts recorded in the present study was more or less similar to that recorded by Rao *et al.* (1965) and Nongbri (1977) although, Damodaran (1974) noticed the incidence as high as 3.7 per cent. Rao *et al.* (1965) stated these cysts to be the remnants of the Wolffian ducts.

Four cases of pyometra were noticed of which two developed in the pregnant animals. In these two cases both the right horns carried the foetus where as the left horns developed pyometra. The other two cases were non-gravid and distended with accumulated pus. (Fig. 2). Histopathological examination of the affected horns showed marked infiltration of polymorphomuclear cells along with few plasma cells. The placenta of the pregnant cases showed caseative necrosis with polymorphonuclear and mononuclear cells infiltration but on Ziehl-Neelsen's stain no acidfast organisms could be detected.

Uterine abscess was one of the other complications caused by infections agents. On the serosal surface a small greyish white foecus was detected and on incision purulent materials could be seen. Microscopical examination, a large numbers neutrophils were noticed along with the eosinophils. (Fig. 3). Caseated exudate was also seen. Bacteriological examination revealed *streoptococcus* spp. Few gram positive cocci were also observed in tissue section when stained by McCullum Good pasture stain, Ziehl-neelson's staining did not reveal any organisms.

The commonest and most important condition recorded was endometritis which was observed in 87 cases and percentage of incidence was as high as 20.23. The endometritis recorded in the study was either haemorrhagic, chronic or diffuse form. Gross changes were noticed in 46 cases which included the presence of brown to black coloured debris in the uterus or large amount of blood tinged fluid or blood tinged or watery mucus. Certain cases could not be classified due to overlapping alterations. Uterine tympany was also noticed in two cases. In addition to these few cases showed presence of few fibrinous strands on the serosal surface of uterus, (Fig. 4), attachment of muscular flap with the carancles and in one cases two carancles were found to be joined with each other (Fig. 5).

Microscopic changes of these cases showed haemorrhagic endometritis which revealed the presence of large numbers red blood cells in the exudate yielded organisms such as *Escherichia coli* and

Pasturella multocida. Chronic form of endometritis were characterised by the infiltration of mononuclear cells and plasma cells in the endometrium, proliferation of connective tissue, with the formation of several small blood vessels. Few cases showed cystic dilatation of the endometrial glands and hyperplasia of mucosal epithelium (Fig. 6). Acute endometritis was evident by desquamation of the epithelial cells (Fig. 7), engorged blood vessels and aggregation of leucocytes around the uterine gland (Fig. 8). Diffused metritis was also noticed by the infiltration of mononuclear cells in all the layers of the uterus.

In this study endometritis was the commonest lesion recorded and it supported the findings of other worker (Sharma *et al.*, 1968; Bhattacharya *et al.* 1970; Rao and Keshavamurty, 1972; Dwivedi and Singh, 1975 and Nongbri, 1977). Hartigan *et al.* (1972) noticed the incidence as high as 50 per cent and *Corynebactrium pyogenes* was isolated from 43 per cent of cases with significant pathological lesions in the endomentrium. Cellular aggregation round the glands within the endometrium were recorded in few cases which were thought to be the important lesions of endometritis in cows (Perkins *et al.* 1954; Kampelmacher, 1954 and De Bois and Van Den Akker, 1957) but Simon and Menutt (1957) were of the opinion that such cellular aggregates may not be of any significance.

Five cases of mucometra were noticed in the present study. The uterus was distended with thick mucus and in all the cases the noticeable charge was that the ovaries were having cystic Graffian follicle. Microscopically there was cystic dilatation of the endometrial glands and in few cases there was hyperplasia of the endometrial epithelium. The mucometra was also reported by the different workers as Kodagali and Kerur (1968), Mukherjee (1972) and Nongbri (1977). Roberts (1971) stated that mucometra might develop following a long standing cystic ovaries asosciated with atrophy of the uterine wall and cystic dialation of the endometrial glands. In the present study also all the cases showed cystic Graffian follicles and in few cases cystic dilatation of the uterine glands which support the view of Roberts (loc cit).

Three cases of melanosis and five cases of haemosiderosis were recorded in the present study. The deposition of black coloured melanin pigment were noticed in the endometrium (Fig. 9). In one case the deposition was so marked that in few places the cellular and architectural details of the endometrium were observed because of the pigments. The section was bleached with 1 per cent potassium permanganate solution indicating the melanotic nature of the pigments. The haemosiderin pigment noticed in those cases in which four cases were accompanied with haemorrhagic endometritis.

Only one case revealed lymphoid hyperplasia in the serosal surface of the uterus. The same nimals showed lymphoid hypernlasia in the ovarian bursa and broad ligament also. Grossly no apparent changes of the genetalia was noticed except that he normal glistening appearance of the whole genitalia was lost. Microscopically focal collection of lymphocytes forming nodules were seen in the serosal surface (Fig. 10). Damodaran (1974) and Sharma *et al.* (1968) recorded this condition in the bursa as well as on the serosal surface of the uterus. The histological picture of this condition was

recorded by Damodaran (1974) who suspected to be a parasitic granuloma and was probably caused by migration of immature forms of *Setaria cervi*. In this study, though few cases of *Setaria cervi* were seen but attempts to find out such correlation did not yield fruitful results.

REFERENCES

Bhattacharya, A.R.; Luktuke, S.N. and Roy, D.J. (1970) *Indian J.* Anim. Sci. **40**: 425.

Burs, D.H.J. (1952) Proceedings of the Second International Congress of Animal Production. p. 175.

Damodaran, S. (1974) Paper presented at Summer Institute in Animal Reproductive Physiology held at Tirupati.

DeBois, L.H.W. and Vanden Akker, S. (1957) *Tijdschr. Diergendacsk.* **82**: 951.

Dwivedi, J.N. (1968) Ph.D. thesis. Agra Univ. Agra.

Dwivedi, J.N. and Singh, U.M. (1975) *Indian J. Anim. Sci.* **45**: 15.

Hartiqan, P.J., Musphy, J.A., Nun, W.R. and Griffin, J.F.T. (1972) *Irish Vet. J.* **26**: 225 (Cited by Vet. Bull) **43**: 1297.

Kampelmacher, E.H. (1954) Second International Congress or Animal reproduction, Copenhagen **2**: 188.

Kodagali, S.B. and Kerur, V.K. (1968) *The Guj. Vet.* **2**: 29.

Mukherjee, S.C. (1972) M.V.Sc. thesis. Orissa Univ. Agric. Tech. Bhube neswar.

Nair, K.P. and Raja, C.K.S.V. (1974) *Kerala J. Vet. Sci.* **5**: 82.

Nongbri, S.S. (1977) M.V.Sc. thesis. Assam. Agril. Univ. Khanapara.

Perkins, J.R., Olds, D. and Seath, D.M. (1954) *J. Dairy Sci.* **37**: 1158.

Rao, A.R.; Narasinha Rao, P. and Rao, A.S.P. (1965) *Indian. Vet. J.* **42**: 751.

Rao Narasinha, A.V. and Kesavamurthy, A. (1972) *Indian Vet. J.* **49**: 61.

Roberts, S.J. (1971) Ind. edn. 2nd edn. Scientific Book Agency, Calcutta.

Sharma, O.P., Bhalla, R.C. and Soni, B.K. (1968) *Indian Vet. J.* **45**: 293.

Simon, J. McNutt, S.H. (1957) *An. J. Vet. Res.* **67**: 241.

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Comparative Study Of Vaginal Cytology Of Ewes During Normal Estrus Cycle And Anestrus

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ABSTRACT

Results of vaginal cytology of 44 ewes (30 cycling and 14 anoestrus) have been discussed. Ewes with normal oestrus cycle showed all types of cells during different phases of oestrus cycle while anestrum ewes showed major percentage of basal cells only.

* * *

Ovine vaginal cytology has been studied by various workers but there is great disagreement on its cell content and variation in normal animals. Vaginal epithelium being responsive to the hormonal stimulation (Sarger *et al.*, 1958), study was conducted to know the response or absence of hormonal response to vaginal epithelium.

Murphy (1924) was first to use vaginal smear technique in cows and reported that vaginal epithelium in cows was not uniform throughout the length of oestrus cycle.

Cole and Miller (1931), Grant (1933-34), Dukes (1955), Radford and Watson (1955) examined vaginal smears from sheep and found thick mucus, leukocytes and a few epithelial cells during pre-estrus. During oestrus, mucus was thin and clear and some cornified cells were present and a few leukocytes could be seen. During Met-oestrus and diestrus mucus was thick, and cheesy, masses of cornified desquamated cells were present and some leukocytes were seen.

Sanger *et al.* (1958) while studying vaginal smears from ewe taken during a normal oestrus cycle reported that in pro-oestrus smear was thin, clear, and cells scarce. All cells were basophilic in staining and were distient and separated. In oestrus, smear contained more mucus. Cells were scarce and of superficial type. During metestrus and dioestrus, smears were thick, sticky white, cheesy and both superficial and parabasal cells were present. Nearly all the cells were cornified. Many had curled edges tor were folded. Some cells showed evidence of degeneration. In pro-oestrus and oestrus, no leukocyte was seen while in meto-estrus and dioestrus, both leucocyte and bacteria were numerous.

Zourgus *et al.* (1977) conducted cytological vaginal studies and examined arborization pheromenon of vaginal mucus to identify cystic oestrus activity in ewe. These authors also studied vaginal smears daily from 3 ewes through two oestrus cycles, and reported that the proportion of basal cells remained low. 10 per cent proportion of intermediate and superficial cells changed accordingly to the stage of the cycle, highest proportion of intermediate cells were found at dioestrus and superficial cells during oestrus.

Table 1 Vaginal Cells during oestrous cycle and anestrus condition.

Sr. No.	Time of study of vaginal cytology	No. of ewes in this category	Average percentage of various types of cells			
			Superficial Squamous	Inter-mediate	Basal	Para-basal
1.	A few hours before oestrus	4	84.0	15.25	0.5	0.25
2.	1-5 days before oestrus	5	9.2	32.8	37.00	21.00
3.	6-9 days before oestrus	7	60.2	28.00	4.7	7.1
4.	10-12 days before oestrus	4	7.00	13.25	42.25	37.00
5.	13-15 days before oestrus	3	5.00	20.33	51.00	23.67
6.	16-17 days before oestrus	7	33.57	52.57	8.43	5.43
7.	Anestrus ewes	14	0.07	0.0	99.00	0.93

Material and Methods

44 ewes of Marwari breed from C.S. W.R.I. farm, Bikaner constituted the material for this study. The vaginal aspirations in sterilized physiological normal saline solution (0.85 per cent NaCl Sol.) were collected from the anterior part of vagina. These aspirations were centrifuged at 3000 rpm for 30 minutes and wet smears were prepared from the sediment. The smears were fixed in methyl alcohol and stained by Giemsa's stain technique.

The vaginal aspirations were collected only once from each ewe but on different days in lots of 10 except the last lot of 14 ewes.

In this study, modern terminology (Symposium on cytological Terminology, 1958) has been used. In all 100 cells were counted in each stained slide. The four type of cells were:

1. Superficial squamous cells.
2. Intermediate cells.
3. Basal cells.
4. Parabasal cells.

Results and Discussion

Out of 44 ewes whose vaginal aspirations were collected, only 30 ewes showed oestrus while 14 ewes were in anoestrus.

After the ewes were detected in oestrus, these were classified into various groups depending upon the number of days the samples were collected before oestrus (Table 1).

As per table 1, group wise cytology of vaginal wall of the ewes was as under:

(1) *A few hours before oestrus:*

These ewes came in oestrus a few hours after the vaginal aspirations were collected. The ewes were bred in estrus by the use of a teaser ram. On an average, during this period, most of the ewes had increased percentage of superstitial squamous cells and a few nuclei showed pyknosis. All cells had rolled edges or irregular shape but nuclei remained distinct, centrally located and well stained. A few cells had undergone complete karatinization.

4 ewes fell under this category.

(ii) *1-5 days before oestrus:*

During this period, intermediate cells and basal cells predominated until pre estrus while superstitial squamous cells got reduced in numbers. In all 5 ewes fell in this category.

(iii) *6-9 days before oestrus:*

Vaginal smears revealed increased percentage of superstitial squamous cells in thick masses, large clumps and files. Most of the cells were evenly stained with centrally located nuclei. Basal cells and parabasal cells decreased in number. There was prominence of folded edges and wrinkled cells and many cells showed evidence of degeneration. Smears revealed heavy infiltration by neutrophils.

7 ewes were recorded in this category.

(iv) *1.-12 days before oestrus:*

On an average, most of the ewes were found to have low percentage of superficial squamous cells and intermediate cells while the percentage of basal and parabasal cells was high.

4 ewes were recorded in this category.

(v) *13-15 days before oestrus:*

On an average, most of the ewes showed decreased percentage of superficial squamous cells and cellular foldings. There was increased percentage of basal and parabasal cells.

3 ewes were recorded in this category.

(vi) *16-17 days before oestrus:*

On an average, most of the ewes showed increased percentage of superficial squamous cells and intermediate cells. 3 ewes had increased percentage of superficial cells while other 4 ewes had increased percentage of intermediate cells. A few basal and parabasal cells were observed.

7 ewes were recorded in this category.

Anestrum ewes:

Vaginal smears of all the ewes numbering 14 in this group revealed major percentage of basal cells and very few parabasal and squamous cells. All cells were basophilic in staining. Scattered neutrophils and abundant bacteria were observed.

The findings of cytological pattern in the present study are in agreement with that reported by Cole and Miller (1931), Grant (1933-34), Radford and Watson (1955) and Sanger *et al.* (1958).

Findings in the anestrum ewes are in agreement with that of Sanger *et al.* (1958).

REFERENCES

Cole, H.H. and Miller, R.F. (1931: Vaginalsmears of ewes Proc. Soc. Expt. Biology & Med., 28: 841. Cited by Sanger et al. (1958). Amer. J. Vet. Res. **19:** 288-294.

Dukes, H.H. (1955): The physiology of domestic animals. 7th Ed. Comstock Publishing Co. Ithaca, N.Y.

Grant, R. (1933-34): Studies on the physiology of reproduction in ewes. Tr. Roy. Soc. of Edinburgh, **58**(1): 1-47.

Murphy, H.S. (1924): Studies of the oestrus or genital cycle of camel. J. Amer. Med. Assoc. **65:** 598.

Radford, H.M. and Watson, R.H. (1955). Changes in the vaignal content of Merino ewes throughout the year. Amer J. Agric. Res., **6:** 431-445.

Sanger, V.L., Engle, P.H. and Bell, D.S. (1958). Vaginal cytology of ewe during oestrus. Amer. Vet. Res., **19:** 283.

Zourgus, A., Neophytou, G. and Elza, K. (1977). Result of cytological studies and examination of arborization phenomenon of vaginal mucus to identify oestrus activity in ewe. Anim. Breed. Abstr. **45**(7): 3311.

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Oestrous Cyclicity In Nali Ewes

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Variation in the length of oestrous cycle and duration of oestrus was reported by Hammond (1944), Sahni and Roy (1967) and Dhanda (1970). The present investigation was undertaken to study the oestrous behaviour of Nali ewes.

The study was based on 89 healthy Nali ewes of 2 to 4 years of age. The Occurrence of behavioural oestrus was determined within 40 to 50 min at 12 hr interval both in the morning and evening by using apronised intact rams. For the accuracy of heat detection the ewes were divided into small groups and the rams were changed after 10 min of teasing. The determination of length of oestrous cycle and the duration of oestrus was made according to Hafez (1952) and Parson and Hunter (1967) respectively. Normal Deviate test of proportions was done to find out the difference between the incidence of oestrus in the morning and evening. The other statistical calculations were carried out according to Snedecor and Cochran (1968).

Among the 4 months, September and October were the peak for the occurrence of oestrus covering about 68% of the total oestrous cycles (Talbe 1). The intense oestrus activity during these 2 months can be utilized for ensuring better breeding programme and managemental control over lambing and lamb crops.

The incidence of normal oestrous cycle (14-19 days) covered 83.7% of the total incidence, and the cycles beyond the normal range were only 16.2%. The percentage of multiple cycles were, however, negligible which attributes to the breeding behaviour of Nali ewes.

The average distribution of oestrous cycle per ewe during the breeding season was 5.82. It was interesting to observe in a few cases the half cycle (8-9 days) with regular rhythmic cyclicity which might be attributed to early regression of corpus luteum (Hafez, 1975).

The mean length of oestrous cycle and duration of oestrus were 17.31 ± 3.3 days and 33.7 ± 0.91 hr respectively, which corroborated with the findings of Sahni and Roy (1967) and Dhanda (1970) in native breeds. The duration of oestrus, however, had wide range of variation (14 to 64 hr) which is similar to those reported by Schindler and Amir (1972) and Taparia (1973).

Variation in the onset of oestrus was observed between morning and evening hours in all the months except in November which may be attributed to small no. of observations during this month. About 65% of the total oestrous cycles commenced in the early morning hours (4 a.m. to 8 a.m.) as against nearly 35% in the evening (4 p.m. to 8. p.m.). The pooled number of incidence of oestrus over all the four months was also significantly ($P<0.01$) more in morning than that of evening hours (Table 1).

TABLE 1. Mean length of oestrous cycle, duration of oestrus, their monthly and diurnal variation

Months	Incidence of oestrous cycles (days)						Length of oestrous cycle	Duration of oestrus	Variation in the incidence of oestrus during morning and evening		
	Single cycle			Multiple cycle							
	Short 14	Normal 14-19	Long 19-26	Double 27-37	Triple 38-57	Total	Mean±S.E. (days)	Mean±S.E. (hrs)	Morning (%)	Evening (%)	Difference
August	2	54	1	—	—	57	17.31+3.3	33.7+0.9	20.36	11.60	8.76**
September	4	108	4	—	—	116			17.31	7.94	9.37**
October	18	92	7	—	—	117			19.34	10.18	9.16**
November	9	35	9	1	1	55			7.12	6.10	1.02NS

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

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REFERENCES

Dhanda, O.P. (1970) *Studies on oestrous pattern of hormonal synchronization of oestrus and subsequent fertility in Nali sheep.* M.Sc. Thesis, Hariyana Agricultural University, Hissar.

Hafez, E.S.E. (1952) Studies on the breeding season and reproduction of the ewe. J. Agri. Sci. **42**: 232.

Hafez, E.S.E. (1975) Reproduction in farm animals 3rd edition. Lea & Febiger Philadelphia pp. 268.

Hammond, J. (Jr) (1944) On the breeding season in sheep. J. Agric. Sci. **34**: 97.

Parson, S.D. and Hunter, C.L. (1967) Effect of ram on duration of oestrus in the ewe. J. Reprod. Fert. **14**: 61.

Sahni, K.L. and Roy, A. (1967) Study on the sexual activity of Bikaneri sheep and conception rate through artificial insemination. Ind. J. Vety. Sci. *37*: 327.

Schindler, H. and Amir, D. (1972) Length of oestrus, duration of phenmena related to oestrus and ovulation time in the local fat tailed Awassi ewe. J. Agric. Sci. Camb. **78**: 151.

Snedecor, W.G. and Cochran, G.W. (1968) *Statistical Methods.* The Iowa State University Press, Ames, Iowa, U.S.A.

Taparia, A.L. (1973) Breeding behaviours in Sonadi sheep. Anim. Breed. Abstr. **41**: 4038.

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Ageing Of Embryos And Foetuses In Bannur Ewes And Surti Does

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ABSTRACT

18 pregnant Bannur ewes and 7 Surti does were used for this study. The embryos and foetuses were procured after slaughter and various body measurements were recorded. The ageing was done by reading the "Normograph" suggested by Cloete (1939). The weight of the foetus and the length of the vertibral column were used as parameters for determining age. The average final appropriate age determined in Bannur ewes from weight and vertibral column length of these foetuses was 24.2 ± 0.00, 46.67 ± 2.05, 67.57 ± 2.54 and 99.53 ± 4.36 days at 1st, 2nd, 3rd and 4th month of gestation period respectively. Similarly in Surti does age was recorded as 36.2 ± 2.49 and 75.13 ± 3.99 days at 2nd and 3rd month of gestation period respectively. Bannur foetuses of 5th month and Surti foetuses of 1st, 4th and 5th month of gestation period were not available for study.

* * *

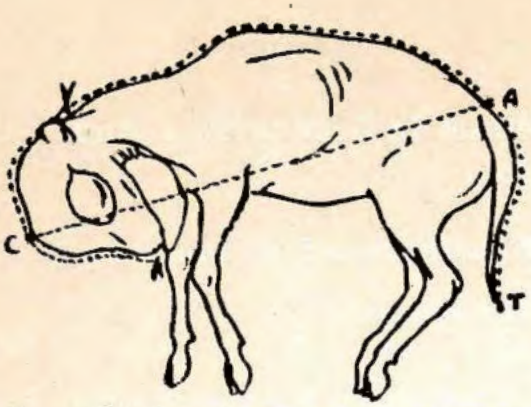
It has been well accepted that growth during foetal period does not differ qualitatively from that subsequent to birth (Cloete, 1939). It is a continuous process starting with fertilization of an ovum which occurs in all body components with the advancing age, prenatal as well as postnatal and ceases with death of the individual. Studies on prenetal development of farm animals have received very little attention. The need for such studies become apparent when evaluation of normal variations is desired in order to make comparative observations between species and to understand the specific effects of environmental factors in development of foetuses in utero. While understanding such type of studies it is necessary to estimate accurately the ages of embryos and foetuses for clinical application in sound animal husbandry practices.

Materials and Methods

Clinically normal gravid uteri from 18 Bannur ewes and 7 Surti does were procured after slaughter irrespective of thier age and parity. On opening the uteri, the embryos and foestuses were removed from the foetal membranes, weighed separately and biometrical observations were recorded by following the technique of Harvey (1959). The length of vertebral column from occipitoatlantic joint to the base of the tail (VR) of all embryos and foetuses was also recorded separately. (Fig.-1).

Technique of Age Determination

In the absence of relevant literature in Indian breeds in respect of ageing of embryos and foetuses, the "Normograph" (Fig. 2) adopted by Cloete (1939) for



1. BCVRT: Total length.
2. CR: Crown rump length in straight line.
3. CVR: Curved crown rump length.
4. VR: Vertebral column length
5. VRT: Vertebral column length, including the tail.

Sheep embryo illustrating measurements used for estimation of age and growth rate of mammalian foetuses.

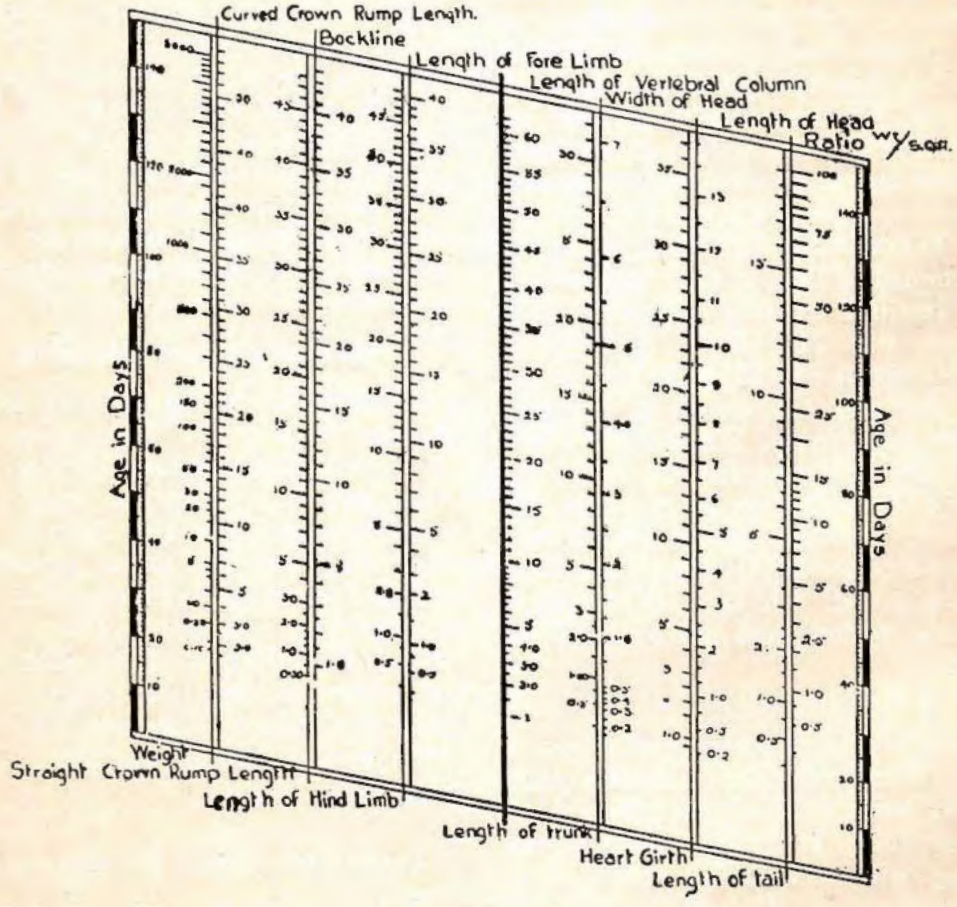


Fig. 2. Normograph for purposes of determining either foetal age from one or more dimensions, or the values of dimensions from these of other dimensions or from foetal age, (From Cloete, 1939. Onderstepoort J. Vet. Sci. and Anim. Ind., 13: 526)

Merino Sheep was used as a standard method for determining appropriate age of unknown embryos and foetuses both in Bannur ewes and Surti does. Assuming the birth weights similar in sheep and goats, the same "Normograph" was used for determination of foetal age in both the species.

The weight of the foetus (Coeff. Var. % 20.07, Maximum amongst all parameters) and the length of vertebral column (VR, Coeff. Var.% 3.81, Minimum of all parameters) were used as parameter for determining the age in days of individual foetuses separately by reading the normograph. Other parameters such as total length of the foetus starting from middle of the nostrils to crown, vertibral column, rump and the tip of the tail (BCVRT), crown rump length in straight line (CR), Curved crown rump length (CVR) and vertebral column length including the tail (VRT) showed the co-efficient of variations % between the limits of earlier two parameters.

After determining the value of dimension the respective scale is marked off lightly by pensil and a thread is stretched on the normograph in such a way so as to lie parallel to the baseline. The intersection points by the thread of the verticle scales on both sides reveal the age in days to be determined.

If the age obtained by the use of weight of the foetus is higher than that obtained from the vertebral column, then the final age would be taken as the vertebral column length value plus one-fifth or the weight value less four-fifths of the difference between these two values e.g.

If the age from weight = X
and age from vertebral column(VR) = Y
and if $X > Y$

then true age $X - 4\frac{(x-y)}{5}$

or $y + \frac{(x-y)}{5}$

Results

All embryos and foetuses were aged by using standard normograph of Merino sheep adopted by Cloete (1939).

The gestation period of ewe and does was divided into five main stages and the embryos and foetuses were grouped according to their age in different stages of gestation period. The 18 foetuses of Bannur ewes were grouped in to only

TABLE 1. Determination of Foetal age using normograph.

No. of Observations	Weight in g.	Vertebral column length Cm.	Age from weight 'X' days	Age from vertebral column 'Y' days	Final Appropriate Age $Y+\frac{(X-Y)}{5}$ days	Gestation period in months
			(A) BANNUR EWES			
n = 3	1.07±0.23	1.50 (n=1)	29.00±1.16	23.00 (n=1)	24.20 (n=1)	First month
n = 6	39.58±9.30	7.67+0.07	52.67±2.68	45.17±1.90	46.67±2.05	Second month
n = 6	279.17±66.17	16.13±1.08	78.50±3.64	64.83±2.27	67.57±2.54	Third month
n = 3	2052.67±398.85	32.49±2.24	119.00±6.51	94.67±4.06	99.53±4.36	Fourth month
			(B) SURTI DOES			
n = 3	6.44±0.34	4.63±0.79	39.67±0.33	35.33±0.37	36.20±2.49	Second month
n = 6	471.25±101.20	19.80±1.94	87.00±4.19	70.50±7.94	75.13±3.99	Third month

four stages of gestation period as none of them was ageing above four months. out of seven Surti does two had twin pregnancy, hence the number of foetuses was nine and none of them was ageing below one month and above three months. Hence these were divided into two groups only. The average observations on ageing of embryos and foetuses at different period of gestation are presented in table 1.

The perusal of above table reveals:

(A) *Bannur ewes:*

The average weight of foetus was 1.07±0.23, 39.58±9.30, 279.17±66.17 and 2052.67±398.85 during 1st, 2nd, 3rd and 4th month of gestation period respectively. The average vertebral column length was 1.5±0.00, 7.67±0.07, 16.13±1.08, and 32.43±2.24 cm. during 1st, 2nd, 3rd and 4th month of gestation period respectively. The average final approximate age determined from weight and vertebral column length of these foetuses was 24.2±0.00, 46.77±2.05, 67.57±2.54 and 99.53±4.36 days at 1st, 2nd, 3rd and 4th month of gestation period respectively.

(B) *Surti does:*

The average weight of the foetus was 6.44±0.34, and 471.25±101.20 g. at 2nd and 3rd month of gestation period respectively. The average vertebral length of foetuses varied from 4.63±0.79 to 19.80 ±1.94 cm during 2nd and 3rd month of gestation period respectively. The average final appropriate age determined from weight and vertebral column length of these foetus was 36.2±2.49 and 75.13±3.99 days of 2nd and 3rd month of gestation period respectively.

Discussions

Winter and Fueffel (1936) divided the prenetal period into ovum, embryonic and foetal period in ewes and linear and circumferential measurements were recorded to understand the prenetal growth. In the present study observations on embryos and foetuses were recorded on similar lines and ageing was done by following a normograph designed by Cloete (1939). Weight and vertebral column length were used as parameters to determine the age. According to their ages the embryos and foetuses were divided monthwise into five different groups. The average age being 24.20±0.00, 46.67±2.05, 67.57±2.54 and 99.53 ±4.36 days in Bannur ewes during 1st, 2nd, 3rd and 4th month of gestation period respectively. Similarly average ages of foetuses of Surti does were 36.2±2.49 and 75.13±3.99 days during 2nd and 3rd month of gestation period respectively. The weights and vertebral column lengths have shown continuous increase with progressing age and they were highly correlated. This is in confirmation with the observations made by Curson & Malan (1935) and Malan and Cursan (1936). Hammond (1927) has also indicated that weight is more variable than the vertebral column length and this has been confirmed in the present study where the weight is more spectacular.

Based on the present studies it is therefore concluded that it is essential to have a large number of pregnant ewes does with known service dates & foetuses of same age group to standardize the normograph of Indian breeds of sheep & goats.

Acknowledgement

The authors feel grateful to Dr. C. R. Sane, formerly Professor of Gynaecology and Dr. S. M. Ajinkya, formerly Associate Dean, Bombay Veterinary College for facilities provided during the research work. The help rendered by the staff members of the Animal Reproduction Department is gratefully acknowledged.

REFERENCES

Cloete, J.H.L. (1939). Prenatal growth in the Marino sheep. endersteport J. Vet. Sci. and Anim. Ind. **13**: 417-588.

Cole, N.N. and Cupps, P.T. (1959). Reproduction in Domestic animals, Vol. I, Academic Press, New York and London.

Curson, H.H. and Malan, A.P. (1935). Studies on sex physiology No. 13, The changing proportion af Marine lamb from 2nd to 5th month of prenatal life, Condertepoort, J. Vet. Sci. and Anim. Ind. **4**: 481-492.

Hammond, J. (1927). The physiology of reproduction in the cow, Cambridge Univ. Press, London.

Harvey, F.B. (1959). Implantation Development of the foetus and foetal membranes, (Cited by Cole and Cupps, 1959).

Malan, A.P. and Curson, H.H. (1936). Studies on sex physiology, No. 15, Further observation from body weight and C.R. length of Marino foetus, Ondersteport J. Vet. Sci. and Anim. Ind. **7**(1): 339.

Winters, L.M. and Fueffel, G. (1939). Minn. Univ. Agr. Expt. Sta. Tech. Bull. No. 118 (Cited by Cole and Cupps 1959).

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The Effect Of Feeding The Silkworm-Fed Mulberry Leaf (Morus Indica Linn) On The Reproductive Status Of Ewes.

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ABSTRACT

Experiments were conducted to investigate the effect of feeding the silkworm-fed mulberry leaf on the reproductive status of cycling and pregnant ewes, and on the uterine weight of ovariectomised rats. Mulberry leaf-fed ewes exhibited oestrus and a low plasma progesterone concentration for a fortnight. Mulberry leaf feeding did not affect the gestation of pregnant ewes. The increased uterine weight of mulberry leaf-fed ovariectomised rats suggest the presence of biologically active phyto-oestrogen in this plant leaf.

* * *

In parts of Karnataka, where dairying and sericulture co-exist, the farmers feed the silkworm-fed mulberry leaf (ML) (*Morus indica Linn*) to their cattle and buffaloes. Although, the mulberry leaf appears to possess the nutritive properties, the conceptional failure and the repeat breeder syndrome appear to occur in mulberry leaf fed animals. This prompted us to investigate the effect of feeding the mulberry leaf to ewes.

Materials and Methods

Experiments in ewes.

Synchronisation of oestrous cycle of ewes: At 12 day intervals, 2 injections of cloprostenol, a PGF_2 alpha analogue (Estrumate, ICI, UK) 20 μg i.m. was given to cycling ewes. Subsequent to this synchronisation, on Day 10 of the oestrous cycle, the ewes were used for the experiment.

Control group: Four cycling (Group I) and 4 pregnant (about 4 months gestation) ewes (Group II) were allowed the routine grazing of the hariyali grass.

Treatment group: Four cycling (Group III) and 4 pregnant ewes (Group IV) were fed the green ML *ad lib* for 15 days.

Both the control and treatment groups were allowed with rams smeared on its brisket with an acriflavine or picric acid ointment.

Blood sampling: Every day, 5 ml blood (5 i.u. heparin/ml blood; Heparin, Biological Evans) samples were collected through the jugular venepuncture. The blood sample was centrifuged at 700 g and the separated plasma was stored at −15°C.

The progesterone concentration in the blood plasma samples was estimated by a radioimmunoassay procedure (Narayana, 1978) at Professor Moudgal's Laboratory, Indian Institute Science, Bangalore.

EXPERIMENTS IN RATS:

Groups of ovariectomised wistar rats (six/Group) were used for the experiments, a week after the ovariectomy.

SOLVENT CONTROL GROUP 1:

To 100 g commercial rat feed (Hindustan Lever, Bombay), 100 ml ethanol was added, mixed, pelleted and dried at room temperature, and fed to rats for a day.

CONTROL GROUP 1.

To 100 g powdered rat feed, 13 μg of oestradiol-17β (Sigma, USA) in 100 ml ethanol was added, mixed, pelleted and dried at room temperature, and fed to rats for a day.

Treatment Group 3:

To 50 g of rat feed, 50 g of dried ML was added and mixed after adding water, pelleted and dried at room temperature, and fed to rats for a day.

All the control and treatment rats were sacrificed on the following day and their uterine weights recorded. The significance of difference of values between the treated and the controls was tested by the analysis of variance (Snedecor & Cochran, 1968), and the values are expressed as mean ± standard error mean.

Results

Experiments in ewes

All the 4 ewes in Group III exhibited oestrus 24 to 36 h after the inittation of feeding of ML. Throughout the course of the experiment, 3 of 4 ewes in this group, exhibited occasional oestrus. Subsequent to this experiment, all the ewes conceived and gave birth to live lambs. In Group IV, despite the exhibition of the behavioural oestrus, neither the gestation length, nor the lambing was affected.

Compared to the control Group I, 3 of 4 ewes in Group III showed a low progesterone concentration during the experimental period. (Fig. 1). In Group IV, despite the expression of oestrus, the progesterone concentration was unaffected and was similar to control Group 2 ewes.

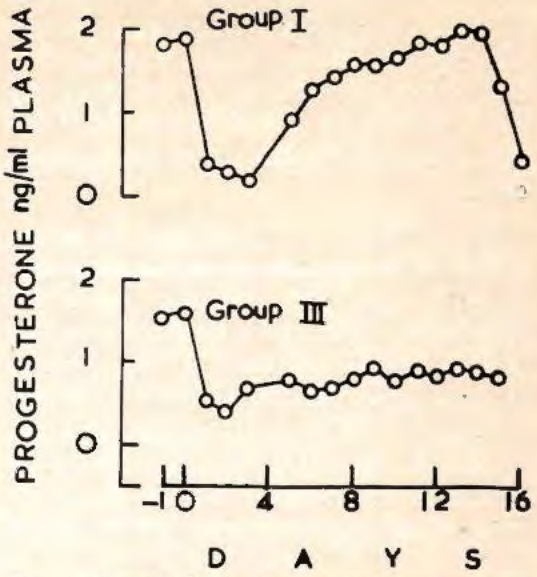

Fig. 1. Jugular blood plasma progesterone concentration in control (Group I) or silkworm-fed mulberry leaf-fed (Group III) ewes. One unresponsive ewe in Group III was not included in this graph. Zero day is the Day of oestrus.

Experiments in rats:

The uterine weight was 80.2±8.9 mg in control Group 1, 95.6±5.4 mg in oestradiol-treated control rats (Group 2) and 124.5±11.6 mg in ML fed rats (Group 3); the uterine weight values of ML-fed rats was significantly ($P<0.05$ by means of analysis of variance; F calculated 4.27) higher compared to non-oestradiol-treated control Group 1.

Discussion

In ewes, feeding ML produced aberrations in the oestrous cycle and a low plasma progesterone concentration. In confirmity with the report of Saksena (1977), the rat bioassay revealed the presence of phyto-oestrogen in this plant

material. The phyto-oestrogen-induced *in vitro* inhibition of progesterone synthesis in bovine granulosa cells (Kaplanski *et al.* 1981) and the luteal phase defects in ewes chronically treated with oestradiol-17β (Robinson, 1982), snggests that the oestrogenic activity of the mulberry leaf accounts for the defective functioning of the corpus luteum of the oestrous cycle.

The occurrence of luteolysis in heifers 25.5 h after the administration of oestradiol-17β and prostaglandin F_2 alpha (Rico *et al*, 1982) and a similar latency to oestrus in ML-fed ewes in the present study suggests that, in addition to phyto-oestrogens, ML could possess prostaglandin-like activity. Other than the report of Elzayat and Stylos (1975) on the existence of prostaglandins in alfalfa, there is no information on the existence of prostaglandins in plants. However, the absence of abortion in ML-fed pregnant ewes reflects either the presence of negligible amount of prostaglandin-like substance, or to the presence of anti-inflammatory principles (Barman et al, 1981) in *Morus indica*.

Acknowledgement

We are grateful to Professor N. R. Moudgal for permitting us to use his radioimmunoassay facilities. We thank Professor R. V. Patil for the encouragement and advice, Dr. S. J. Seshadri, Director, for his co-operation and encouragement, Professr A. V. Rai for the permission to use the ewes and the staff of Sericulture Department, UAS, for providing us the mulberry leaf residue.

REFERENCES

Barman, T.K., Chatterjee, G.K., Nag Chaudhuri, A.K. and Pal, S.P. (1981) Some pharmacological actions of the extract of *Morus indica* Linn roots. *Indian Journal of Pharmacology*, **12**: 237-242.

Elzayat, S and Stylos, W.A. (1975) Identification of prostaglandins (PG) A_1, E_2, F_2alpha and/or PG-like materials in commercial animal feeds and their significance. *Federation Proceedings*, **34**: 791.

Kaplanski, O., Shemesh, M. and Berman, A. (1981) Effects of phyto-oestrogens on progIesterone synthesis by isolated bovine granulosa cells. *Journal of Endocrinology*, **89**: 343-348.

Narayana, K. (1978) Neuropnarmacology of LH ad FSH secretion in the ewe. Ph.D. thesis, Univesrity of Liverpool, U. K.

Rico, L.E., Thatcher, W.W., Drost, M. and Grimmett, J. (1982) Effect of oestradiol-17β on PGF_2alpha induced luteolysis. *Journal of Dairy Science*, **65**: Suppl. 1. 181-182.

Robinson, T.J. (1982) Hammond memorial lecture: the magic of Hammond. *Journal of Reproduction and Fertility*, **66**: 397-410.

Saksena, S.K. (1977) Effect of plant oestrogen on growth of mice. *Indian Veterinary Journal*, **54**: 558:560.

Snedecor, G.W. and Cochran, W.G. (1968) *Statistical Methods* Oxford & IBH, Calcutta.

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A Study On Dystokia, Retained Placenta And Foetal Malformations In Muzaffarnagari Sheep

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ABSTRACT

Incidences of dystocia, retention of placenta and foetal anomalies in Muzaffarnagari breed have been recorded in the order 2%, 4.06% and 4%. Details on foetal anomalies and dystocia have been presented and discussed.

* * *

The present studies were made at the Sheep Farm of the Indian Veterinary Research Institute, Izatnagar. observations on 200 parturitions were made in Muzaffarnagari breed of sheep which have been presented in this study.

Results and Discussion

During the period of the study 4 cases (2%) of difficult parturition were recorded. The difficulty in parturition was due to the posterior presentation of foetus in one animal. In two ewes their heads were retained beneath the chest resulting in dystocia. in one animal the cause of dystocia was due to the lateral deviation of the head and retention of the both forelegs in the birth canal. (Table 1).

The correction of the dystocia in the above mentioned 4 cases was done manually by mutation process and the lambs were delivered by gentle traction. In each case it was a single birth.

In one case after the extraction of the foetus it was observed that the lamb had a curved vertebral spine having a deep depression at the loin region. This was recognised as an anomaly of development.

TABLE 1 Incidence of dystocia, retention of foetal membranes and foetal malformation in sheep.

Item	No. Observed
1. Dystocia	4
Due to:	
(i) Posterior presentation	1
(ii) Retention of head beneath the chest	2
(iii) Lateral deviation of head	
2. Retention of placenta (amongst 197 normal parturitions)	8
3. Abnormal foetuses	8

According to Roberts (1956) the incidence of dystocia in cattle is 3.3% and in horses 1.1%. Naaktgeboren and Stegman (1968) in their observation in Texas sheep observed 77.3% of the ewes lambing naturally. When the data on 1237 births were analysed Aamdal and Lyngset (1970) found the incidence of dystocia as 24.3% in Dala, 26.5% in Rygja, 18.2% in Cheviot, 7.9% in Steiger and 2.6% in Old Norwegian breeds of sheep. From these reports it appears that the incidence of difficult parturition in the breed under present study is almost at par with Old Norwegian and it is very low as compared to the incidences in other breeds. One possible explanation of lower incidence of dystocia in the present study may be that the present

TABLE 2. Details of foetal malformations

Sr. No.	Ewe No.	Stage of gestation (days)	Sex of lamb	Weight of lamb (kg)	C-R length (cm)	Description of the abnormalities	Type	Remarks
1.	677	—	F	0.38	20	Upper jaw was longer than the lower jaw	Brachygnathia or parrot-mouth	born Dead
2.	598	—	M	3.5	44	Complete absence of lower jaw and tongue	Agnathia	Born alive but died after 20 minutes
3.	464	—	M	1.9	34	Lower jaw was shorter than the upper jaw	Brachygnathia	born Dead
4.	476	—	M	1.2	29	Upper jaw was shorter than the lower jaw		,,
5.	695	144	M	1.2	30	(i) — do — (ii) Absence of both the eyes (iii) Absence of nose (iv) Hydrocephalous	Pig-mouth ,,	,,
6.	18	156	F	2.7	41	Curved vertebral spine (unable to stand)		Born alive died after 1 day
7	177	153	F	2.6	42.5	Curved vertebral spine (unable to stand)		Born alive died after 3 days
8.	454	—	M	2.85	44	Both the posterior legs are much enlarged unable to stand at all	Deformed limbs	Born alive died after 2 days

study is exclusively on pluripara ewes where the incidence of dystocia is usually low (Roberts, 1956) and the twinning incidence in this breed is also very low (4.5%), the condition which favours the occurrence of dystocia (Roberts, 1956; Arthur, 1964 and Verma *et al.*, 1973). It is also apparent from the reports of Roberts (1956) that the incidence of dystocia in large animals like cattle and horses, is low. The incidence of twinning in mare is about 0.5 to 1.5% and in cattle it is 1.04% (Roberts, 1956). In this study no case of dystocia due to twinning was recorded rather the cause of dystocia was due to abnormal presentation, position, or posture of the foetus.

In all, 11 cases of retention of placenta were recorded during the tenure of the study among 200 observations on parturition. Out of these 11 cases, in 3 ewes the placenta were retained after the delivery of the foetuses having anatomical deformities. Thus, the extent of the retention of placenta without any apparent reasons was observed in 8 animals out of 197 normal lambings i.e. 4.06%. A total number of 8 cases were recorded on the anomalies of foetal development out of which in 3 cases the placenta were retained after the birth of the lambs.

Palmer (1932) observed 11.7% cases of retention of placenta in cattle while the extent has been noted to be upto 8.3% (Kenedy, 1947), 6.4% (Boyed and Sellers, 1948), 8% (Vandeplassche and Martens, 1961), 4 to 5% (Arthur, 1964) and 1.96% (Moller *et al.*, 1967). The occurrence of retention of placenta as recorded in the present studies in ewes is higher than the report of Moller *et al.* (1967) but it is very close to the observation in cattle. Other reports in cattle show much higher figures (Palmer, 1932;

Kenedy, 1947 and Vandeplassche and Martens, 1961). According to Craig (1952) the retention of placenta is less frequent in ewes than in cows.

During the course of the present study 8 lambs were observed to possess anatomical deformities showing some developmental anomalies, Photograph I. The details of the anomalies have been preseented in Table II.

Photograph I

Brachy-gnathia

The reports to which extent the foetal anomalies in sheep occur are lacking, however, the conditions observed in this study have also been reported and described by Muir *et al.* (1937), Kelley (1942), Rae (1956) and Singh *et al.*(1970).

Anomalies of foetal developments have been recognised to be a hereditary character governed by recessive gene (Kelley, 1942 and Roberts, 1956). From the genetic and economic point of view these conditions are important since they are recognised of being inherited and result in lamb losses. Elaborate studies therefore, are needed to establish the definite mode of inheritance of these characters.

Acknowledgement

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REFERENCES

Aamdal, J. and Lyngset, O. (1970). Dystocia in omr sheep breeds. Norsk Vet. Tidsskr., **82**: 82-85. (Anim. Breed. Abstr., **39**: 3444)

Arthur, G.H. (1964). Wright's Veterinary Obstetrics, Bailliere Tindl. and Cox., London.

Boyed, W.L. and Sellers, A.F. (1948). Some observations on post-parturient cows in four separate herds as related to expulsion of their foetal membranes. Cor. Vet., **38**(3): 263.

Craig, J.F. (1952). Flemings Veterinary Obstetrics. Bailliere Tindall and Co., London.

Kelley, R.B. (1942). Inheritance of a short lower jaw. J. Council Sci. Indust. Res., **15**: 189.

Kenedy, A.J. (1947). Retention of the placenta in the bovine. Vet. Rec., **59**(38): 519.

Moller, K., Newling, P.E., Robson, M.J., Janseh, G.J., Meursinge, J.A. and Cooper, M.G. (1967). Retained foetal membranes in dairy herds in the Huntley district. N.Z. Vet. Jour., **15**(7): 111.

Muir, G.W., Deakin, A., McMillan, A.A. and Freeborn, S.G. (1937). Bulldog jaw and parrot-mouth defects of sheep. Pub. Canada Dep. Agric., **588**: 7. (Anim. Breed. Abstr., **6**(3): 210.

Naaktgeboren, C. and Stegman, J.H.J. (1968). Unaided parturition in primiparous Texel ewes. Tijds-Chr. Diergeneek, **93**: 745-756 (Du with Eng., Fr., Ger. Sp. Summs), Zool. Lab. Univ. Amsterdam. (Anim. Breed. Abstr., **36**(4): 3757).

Palmer, C.C. (1932). Clinical studies on retained placenta in the cow. J.A.V.M.A., **80**(1): 59.

Rae, A.L. (1956). Brachygnathia, overshort or parrot mouth in sheep. Advances in Genetics, 1956, **8**: 190.

Roberts, S.J. (1956). Veterinary Obstetrics and Genital Diseases. Published by the author, Ithaca, New York, First Ed., Edward Brothers Inc. Ann. Arbor., Michigan.

Singh, V.K., Mathur, P.B. and Chattopadhyay, S.K. (1970). Brachygnathia in sheep and its inheritance. Curr. Sci., **39**(8): 187-188.

Vandeplasche, M. and Martens, C. (1961). The influence of oestrogens on length of gestation and on retention of the placenta in dairy cattle. Proc. IV Internat. Cong. on An. Reprod., The Hague, III: 671.

Verma, S.K., Tyagi, R.P.S., Gera, K.L. and Khanna, B.M. (1973). A case of foetal dystocia due to twins. Indian Vet. J., **50**(3): 287.

IJAR 5: 1: 107-109 1984

MACRO CAPSULES

An Efficient Oral Drug Delivery System For Veterinary Use

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ABSTRACT

A brief review of different oral drug delivery systems adopted by Veterinarians reveals the limitations encountered in drug administration through Tablets, Bolli, Drenches and Paste. The oral drug delivery system using **Hard Gelatin Capsules** as drug containers affords distinct advantages in terms of accuracy and consistency of the drug dosage along with minimal wastage and microbial contamination.

* * *

The commonly practiced methods of drug administration for proprietory as well as compounded and dispensed drugs by pharmacies on veterinarian's prescriptions are in the forms of Tablets, Bolli, Drench or Paste. The tablets and bolli are normally available in limited fixed dose formulations. Hence proper dosing necessiates breaking the tablet in accordance with the prescription, with practically no assurance on uniform distribution of the drug within the entire tablet. Liquid drenching using drenching bottles is commonly used for administering syrups. However, this method is cumbersome in case of suspensions and is messy as well as time consuming. Also one is faced with the risk of the fluid entering the wind pipe which can lead to unwarranted pneumonic complications. Sophisticated drenching guns are not easily available to farmers with a small number of animals. Paste formulations necessiate use of special paste guns and a considerable quantity of drug is lost during the dosage preparation. One of the most commonly used methods of drug administration is in the form of admixture with feed, but the major limitation is inaccuracy of drug availability, wastage and inconsistent drug intake.

Hard Gelatin Capsules as a preferred dosage form has been well received and accepted by the pharmacist for medicinal use covering both human as well as Veterinary Treatment. This is because the use of Hard Gelatin Capsules ensures high accuracy of dosage without any wastage and ensures the prescribed drug dosage availability. However, the use of Hard Gelatin Capsules for Veterinary Medication had not been practiced extensively on account of lack of availability of large capsules of different sizes having approved physical, chemical and microbial characteristics.

Materials and Methods

Hard Gelatin Capsules:

Hard Gelatin Capsules with different

sizes and capacities were manufactured using pharmaceutical grade gelatin, edible colours and preservatives. The individual ingredients as well as the finished products were tested for critical physical dimensions, chemical and microbiological tests. The details of the conformance of the hard gelatin capsules with the required micro-biological standards are presented in Table 1.

TABLE 1 Micro Biological specifications of Macro Capsules

Size	Total Bacterial Count	E Coli	S. Typhosa
'7'			
'10'			
'10.5'	Less than 1000 Colonies		
'11'	per Gram		
'12'			
'12.5'			
'13'			
'12.5'		Absent	Absent
'13'			

The Hard Gelatin Capsules were filled with Lactose and the invitro disintegration time according to U.S.P. was found to be less than 15 minutes.

Animal Experiments:

For the purpose of experiment 30 dairy cows with weights between 200 and 350 kgs. were taken as subjects. Hard Gelatin Capsules filled with 20 and 10 gms. of lactose were administered using a stainless steel piston type balling gun (The administration of filled hard gelatin capsules into the mouth by hand was found to be not practical through for intra-uterinary therapy, the hand can be conveniently used). These field trials were conducted in our Research Farm House.

Results:

The Hard Gelatin Capsules filled with 20 and 10 gms of lactose slipped down the gullets of the animals without any discomfort on account of the cylindrical shape and slippery surface of the capsules. Blockage or choking of the respiratory as well as G.I. tract was not observed in any animal. The extraneous microbial contamination of the drug was minimised on account of its easy filling procedure.

A consistent accuracy of dosing was achieved on account of selecting the appropriate capsule size.

Discussion

The administration of drugs in the prescribed dosage form for animals can be easily achieved by use of capsules. The gastro-intestinal availability of the drug for the animals is assured on account of the low dissolution time of the hard gelatin capsules.

The probability of extraneous microbiological contamination during the drug administration is minimised since the capsule forms an inert protective cover around the active drug substance.

The accuracy of the prescribed dosage is guaranteed on account of the inherent volumetric accuracy of the drug container i.e. the capsule.

The administration of capsules to the technique except a simple stainless steel piston type balling gun.

From the above observations, it is apparent that using Hard Gelatin Capsules for the administration of drugs to the animals offers distinct advantages over other methods of drug administration.

In Veterinary practice, especially in cattle, use of anthelmintics is made as a routine once in 6 months, to eradicate worms.

In big cattle herds, when medication is done either in the form of powder, bolus or in the form of adrench, great uncertainty is always experienced as regards the accuracy of dosage, and if it would reach the stomach in proper quantities. Moreover, it is difficult to dose a number of animals by hand alone, either for drenching, or introduction of bolus in the throat by bare hands in each and every animal.

A trial was undertaken at the C. B. Farm, Kandivli, to ascertain use of capsules in which the very effective anthelmintic "THIABENDOL" was stuffed, the total quantity being 20 gms. This was administered by means of stainless steel guns.

This method of administration was found very effective. There was ease af administration, no spoilage at all due to the capsule, no injuries to the hands of the attendents, and the guns were easy to sterilise. Moreover, there was a great satisfaction that adequate quantities reached the stomach to produce desirable effects.

The use of capsules is therefore highly recommended, when mass-administration of medicaments in the powder form are to be dosed.

Acknowledgement

The authors would like to acknowledge the technical assistance provided by M.S.R. Foundation, Bombay, and The Associated Capsules Group of Companies at Kandivli West, Bombay-400 067, India, for developing the technology and supplying different sizes of Macro Capsules per requirements for the above studies.

IJAR 5: 1: 110-112 1984

SHORT COMMUNICATIONS

A Case Of Suppurative Orchitis In A Buffalo Bull

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Deshpande

Clinical andrological investigation of buffalo bulls to evaluate them for reproductive soundness before they are put to service and then periodically as well, is very essential especially in commercial dairy herds in order to keep the dairy industry as a profitable enterprise. Otherwise many a times, attention of herdsman is diverted towards the bull at a very late stage when sufficient damage has already been caused resulting in lowering the over all conception rate in the herd following natural service. One such bull was reported to us for investigation in September, 1983.

History:

A Murrah buffalo bull aged 3½ years was used for natural service since six months at one of the Bombay stables (Fig. 1). The bull was farm bred, well built and was having strong sexual behaviour. Twenty buffaloes served by the bull during the period of three months prior to investigation were found non-pregnant.

Clinical Findings:

1. *Andrological Investigations:*

The bull was having excellent serving ability and serving behaviour. Testicular size was abnormally enlarged however, there was no pain on palpation. The testicular tissue was felt soft, flabby with fluctuating fluid inside but the form and countur of testes was maintained (Fig. 2).

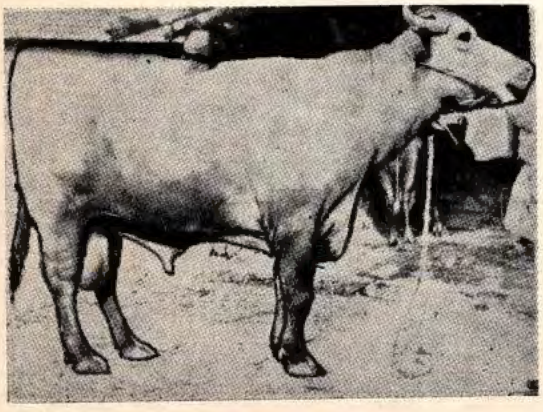

Figure 1. Buffalo bull showing pendulous orchitis testes.

Figure 2. Buffalo bull showing the flabby consistency of testes indicating fluid inside.

The testicular measurements (cm) were as follows:

	Length	*Breadth*	*Circumference*
Left testis	29.00	12.00	48.5
Right testis	27.5	13.00	

The accessory sex apparatus i.e. ampullae, prostate, seminal vesicles and penis were found to be rather normal on examination.

2. *Semen Evaluation:*

Semen collections were obtained at weekly intervals for 3 times. Four successive ejaculates were taken at each time. The average values were as follows:

Volume-1.8 ml., Colour-Watery to cloudy, Consistency-Thin, Density-0, pH-6.8 to 7.0, Mass activity, initial motility-flat, Live spermatozoa-0%, Total sperm count-Aspermia.

3. *Semen Culture:*

Semen samples were cultured for isolation of bacterial organisms. It was positive for infectious organisms staphylococcus Pyogenes (Var. aureus) and E. Coli.

4. *Blood serum examination:*

Blood serum of the bull was sent for the test of Brucellosis on two occasions. The laboratory test revealed a positive titre (1:160) in serum agglutination testing for brucellosis.

Testicular pathology due to acquired causes is much more common than congenital or hereditary and these include testicular degeneration, orchitis, fibrosis and calcification. Lagerlof and Co-workers estimated that 75 to 80% of testicular pathology is related to testicular degeneration including firbosis and orchitis. The epithelium of seminiferous tubule is highly sensitive to any adverse influences with resulting marked effect on spermatogenesis. The testicular measurements observed in the present case are nearly double as compared to the work of Joshi, et al (1967) who reported the normal length, breadth and circumstances for buffalo left testis as 11.19, 4.62 & 12.00 cm and for right testis as 11.21, 4.57 & 12.00 cm respectively. The damage to the seminiferous tubules in the present case was very extensive which was noted by watery semen ejaculates with total aspermia. The sexual behaviour of the bull was not disturbed.

In bacterial diseases localizing in the testis, abscessation may occur. Infectious agents resulting in orchitis are Brucella abortus both field strains and strain 19 in bulls (Lambert, et al, 1964). The high blood serum titre positive for brucellosis in the present bull indicates the possible cause of orchitis. Whereas, isolation of pus forming suppurative organisms (Staph. Pyogenes) from the semen culture certainly revealed that these organisms might have been voided from the diseased orchitis testes to the semen ejaculate. This infection might have set in the testes unknowingly simultaneously with orchitis due to Brucella organism for a long time as there was no acute pain or inflammation of the testes. Sporadic infection of the testis with staphylococci, streptococci, E. Coli, Proteus and Pseudomonas organisms have been reported as a cause of orchitis in male domestic animals (Roberts, 1971). The possibility of scrotal hydrocele, haematocele in the present bull was ruled out as the fluid was not inbetween testes and scrotum.

A rare case of suppurative orchitis in a buffalo bull is placed on record.

REFERENCES

Joshi, N.H.; Luktuke S.N. and Chatterjee, S.N. (1967) Studies on the biometry of reproductive tract and some endocrine glands of the buffalo male. Indian Vet. J. **44**: 137-145.

Lambert G., Deyoe, B.L. and Painter, G.M. (1964) Postvaccinal persistance of Brucella abortus strain 19 in two bulls. J. Amer. Vet. Med. Asso. **145**-909.

Roberts, S.J. (1971) Veterinary Obstetrics and Genital Diseases 2nd Edn. Ithaca, New York pp 674.

IJAR 5: 1: 113-114 1984

Distribution Of The Lymphoid Tissue And Lymphatics In The Broad Ligaments Of Surti Buffalo

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ABSTRACT

Routine paraffin sections cut at 8-10 microns and stained with H. & E.; obtained from the broad ligaments and uterus of 35 non-pregnant and 10 pregnant (1st trimester) Surti buffaloes; revealed no lymph nodes in the broad ligaments. However, several solitary lymph nodules and few aggregations of the same were observed. The lymphatic plexus present in the myometrium,continued between the smooth muscle layers of the broad ligaments and constituted a part of the vascular layer. Endometrial lymphatic plexus could not be observed either in the uterus or extending into the broad ligaments. These lymphatics had numerous valves. The lumen of the lymphatics was enlarged during pregnancy. This information could be of use in the investigations on the reproduction.

* * *

There is scanty work on the lymphoid tissue and lymphatics of the buffalo (Vyas and Mariappa, 1970; Barowal and Dhingra, 1978, Vyas and Mudholkar, 1972). Bagi and Vyas (1983) have studied the structure of broad ligaments in non pregnant and pregnant Surti buffalo. As there was no work on the distribution of lymphoid tissue and lymphatics of the broad ligament in the buffalo, the present investigation was carried out.

Materials and Methods

Uterus along with their both the broad ligaments from 35 non pregnant and 10 pregnant (1st trimester of pregnancy), were obtained immediately after slaughter from the local abattoir. The broad ligaments were searched for the lymph nodes. Tissue were taken from three places viz., mesovarium, mesometrium at the attachment with body wall and mesometrium attached to the uterus. They were processed to cut paraffin sections at 8-10 microns which were stained with Harris (Luna, 1968) haemetoxylin & eosin.

Results and Discussion

Gross examination could not reveal any lymph node. However, on microscopic examination, several solitary nodules and few aggregations of the same were observed in the mesovarium region.

The lymphatic plexus present in the myometrium continued in the broad ligaments between the muscle layers and thus, it constituted a part of the vascular layer of the uterus as well as that of the broad ligaments. This is in agreement with the picture in the ox described by Dyce and Wensing (1971), Sack (1973) and Dellman and Brown (1976). The lymphatic plexuse present in the endometrium of the monkey and the mare

as described by Yoffey and Courtice (1970) could not be observed either in the endometrium or as its possible extension into the broad ligaments of the buffalo under study with H & E stained sections. Endometrial plexuses is mentioned to be absent in Merino sheep (Sass, 1964).

All the lymphatics of the vascular layer possessed numerous valves. The lumen of the lymphatics during pregnancy, was enlarged. This is in agreement with the description given by yoffey and Courtice (1970).

Acknowledgement

Thanks are due to Dr. M. R. Patel, Principal of the College for providing the permission and all the facilities.

REFERENCES

Bagi, A.S. and Vyas, K.N., 1983. Structure of the broad ligaments during non-pregnant State and first trimester of pregnancy in the Surti buffalo. Indian Vet. J. 60. August, 1983: 619-622.

Barnwal, A.K. and Dhingra, L.D. 1978. A note on the lymphatic drainage of mammary gland of buffalo. Indian J. Anim. Sci. 48(10): 753-755.

Dellmann, H. and Brown E.M., 1976. Text book of Veterinary histology. pp. 163-184. Lea and Febiger, Philadelphia.

Dyce, K.M. and Wensing, C.J.G., 1971. Essentials of bovine anatomy. pp. 106-145. Lea and Febiger, Philadelphia.

Luna, L.G.Ed. 1968. Manual of histologic staining methods of the armed forces institute of pathology. 3rd Edn. McGraw-Hill Book Co., New York.

Sack, W.O. 1973. The viscerna of the domestic mammals. pp. 351-389. Verlag Paul Parey, Berlin and Hamburg.

Sass, M.B. 1964. Lymphatic system of the reproductive organs in pregnancy. Ph.D. Thesis, Australian National University, Canberra. (c.f. Yoffey & Courtice, 1970).

Vyas, K.N. and Mariappa, D., 1970. Distribution of lymphoid tissue in the ileum of the Indian Buffalo (Bos bubalis) The Indian Vety. Journal, **47**: 487-490.

Vyas, K.N. and Mudholkar, D.R., 1972. On the distribution of the lymphoid tissue in the alimentary tract of the Indian buffalo (*Bos bubalis*). Gujvet. 6-2: 30-32.

Yoffey, J.M. and Courtice, F.C., 1970. Lymphatics, lymph and the lymphomyeloid Comples. 2nd Ed. Academic Press. N.Y. London. pp. 225-266.

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Spontaneous Vaginal Rupture At Parturition In A Buffaloe

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ABSTRACT

A case of spontaneous vaginal rupture at parturition in a buffaloe is reported. Pelvis anatomical variations and parturient straining can predispose for spontaneous vaginal rupture in a buffaloe, is discussed.

* * *

Spontaneous vaginal rupture is uncommon during parturition in mammals. The review of literature reveals that non-spontaneous vaginal rupture occurs due to improper presentation, position and posture of the foetus in Equines and bovines (Roberts, 1971); faulty obstetrical techniques in farm animals (Arthur *et al.*, 1982), excessive traction of a normal presented foetus in bovines (Roberts, 1971), improper embryotomy in heifers (Benesh, 1954), and due to layman manupulations of dystokia in bovines (Roberts, 1971; Arthur, *et al.*, 1982). Whereas there are no reports on spontaneous and non-spontaneous rupture of vagina at parturition in buffaloes. Hence, a case of spontaneous vaginal rupture at parturition in a buffaloe is reported.

Case report and discussion

A non-descriptive breed, full term, pluriparous buffaloe with symptoms of parturition for the last twenty hours, was brought to the Veterinary College Hospital, with the history of perinial swelling and bleeding from the vagina. The patient had an abnormal counter of vulval lips. Pervaginal examination revealed the presence of a dead foetus in normal postero-dorsal presentation with the hind lims passing through the ruptured vaginal vent and were present below the rectum. There was transverse rupture on the dorsal wall of vagina, just posterior to Portio-vaginalis. The partially attached cervix was draged anterior to the pelvic brim. There were clots of blood in the vagina and raw blood was oozing from the ruptured region. After epidural anaesthesia and Haemostat parentral administration, the foetus was repelled intra-uterine till the fet-locks were drawn out of the ruptured vaginal vent and brought into vaginal canal. By simple traction the foetus was delivered. The blind technique of suturing the vaginal vent with two hands pervaginally was adopted using Integron cat-gug No. 3, Fluid and Antibiotic therapy was followed by expultion of placenta on the next day. Parentral and local antibiotic therapy was adopted for the succeeding five days with success.

The anatomical variations in buffaloe pelvis can predispose for spontaneous vaginal rupture at parturition. In Murrah buffaloes, Pectinoiis Pubiss very sharp, Pelvic inlet is placed verti

cally. Pelvic roof is compressed posteriorly, last sacral vertebra is loosely attached to the Sacrum and Pubis, and the dorsal line inclines downward from Sacral region (Hadi and Sane, 1965). Whereas in exotic breeds of cow, Pectomosis pubis is blunt, Pelvic inlet is placed oblique, pelvic roof is less compressed posteriorly, the last sacral vertebra is firmly attached to sacrum, and the dorsal line is nearly straight even at Sacral region (Sisson and Grossman, 1964). These pelvic anatomical variations casue more elevation of foetus while it is getting into pelvic cavity during parturition and the foetal limbs are directed more towards the compressed Pelvic roof in buffaloes than in cows. Further, the parturient straining was the immediate cause of spontaneous vaginal rupture in pregnant ewes with twins, (White, 1961). In the present case there was no artificial interference in parturition and probably these pelvic anatomical variations and parturient straining could have caused the spontaneous rupture of the dorsal wall of vagina.

REFERENCES

Arthur, G.H., Moakes, D.E. and Pearson, H.G.,1982. *Veterinary Reproduction and Obstetrics*, 5th Edn. Bailliene Tindall, London, p. 237-238.

Beneset, F. 1954. Treatment of perforation in the vaginal wall resulting flown embryotomy in a heifer. Wein. tierazt. Masche, **41**: 589-593.

Hachi, M.A. and Sane, C.R. 1965. Critical observation on certain anatomical features encountered during study of female murrah buffaloe pelvis. Indian Vet. J. **42**: 327-330.

Roberts, S.J., 1971. *Veterinary Obstetrics and Genetal Diseases*, 2nd Edn., CBS. Publishers and distributors India. p. 307-308.

Sissons and Grossman, J.D., 1964, *The Anatomy of Domestic Animal*. 4th Edn. W.B. Saunders Co., Philadelphia.

White J.B., 1961. An unexplained condition in pregnant ewes. Vet. Rec., 71: 281-330.

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Varicosity Of The Cervical Veins In a Cow

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ABSTRACT

A case of varicosity of cervical veins in a cow and its surgical treatment is reported.

* * *

Varicosity of the cervical veins in bovines is a comparatively rare condition. The present report places on record a case of varicosity of the cervical veins in a cow.

Case History

An eight year old pleuriparous cow had a pedunculated growth projecting from the vulval lips. The cow was presented for gynaecological examination in an infertility camp at Atholi, Calicut in June 1982. The growth was first noticed at the vulval lips by the owner a month back. This was found to enlarge and elongate as the time passed (Fig. 1). The cow was reported to have a normal calving four months back, but did not yet exhibit any oestrum.

Observations

The general condition of the animal was poor. The growth protruding through the vulva was 5 cm. long on the outside, cylindrical in shape with the tip being reddish and spherical. The animal evinced slight pain during urination. Per rectal examination revealed that the growth had originated from the cervix. The cervix was slightly thickened and enlarged. The uterus appeared normal in size but flabby. Both the ovaries were smooth and flat and did not show any indication of ovarian cyclical activity. On vaginal

examination, the hand could be passed with much difficulty upto the cervix. The growth was pedunculated with a cylindrical stalk 2 to 3 cm. in diameter with a spherical tip. The free end of the mass showed prominent varicose blood vessels.

The mass was surgically removed and the biopsy material was subjected to

histopathological study. The vulval lips and the mass protruding outside was washed with potassium permanganate (1:1000) solution. Caudal epidural anesthesia was given with 2% procaine hydrochloride. The cervix was pulled out using a valsaleum forceps and a firm tourniquet was applied close to the cervix, 5 mts. after inducing local anesthesia. Another ligature was applied close to the tourniquet and the growth was incised in between. The cut end was cauterized and the tourniquet was removed. The cervix was replaced to normal position. The animal was given penidure LA 48 on the day of operation.

Fig. 2. Picture showing many markedly dileted veins embedded in fibrous tissue stroma, Vessels showing thrombosis (H & E×100)

Tissue pieces of 0.5 cm. size both from the stalk and the tip of the growth were processed for histopathological studies (Humason, 1972).

The histopathological picture showed many markedly dilated veins embedded in a fibrous tissue stroma. Most of the vessels showed thrombosis (Fig. 2). There was abundant fibrovascular stroma around the varicose veins. There was moderate degree of hypertrophy of the vessel wall. The cord like protrusion of the mass, the presence of thrombosed vessels on gross examination and the histopathological evidence of mature veins with hyperplastic wall filled with blood suggest the varicosity of the veins.

Varicose veins are those which are markedly dilated and elongated and they follow an irregular tortuous course in order to accommodate the excess length (Smith *et al* 1972). Initially, they will hold an abnormal amount of blood and later on there will be hypertrophy of media from increased strain, followed by atrophy and replacement fibrosis. The varicosity of cervical veins in the present case might have resulted from traumatic factors caused at the time of last calving. The hindrance of return blood flow at some point in advance of the injured area might have caused this condition.

REFERENCES

Humason, G.L. (1972) *Animal Tissue Techniques*. W.H. Freeman and Co. Sen Francisco 3rd ed. pp. 551-554.

Smith, H.A., Johes, T.C. and Hunt, R.D. (1972) *Veterinary Pathology* & Lea & Febiger, Philadelphia 4th ed. p. 1162

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Electrophoresis And Gel Filtration Of Normal And Frozen Buffalo Semen

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ABSTRACT

The electrophorotic and gelfiltration pattern of buffalo semen pre and post freezing has been reported.

* * *

With the introduction of deep frozen semen in Cattle Breeding it was also a need of the time to investigate the application of freezing technique to buffalo semen and also to investigate the effect of freezing on buffalo semen.

The present investigation has been carried out to see the suitability of citric acid whey with Glycerol as diluent (Ganguli *et al*, 1973 and Bhosrekar and Ganguli 1976) for freezing buffalo semen. The studies reported herein concerned with the electrophorotic and Gel filtration of buffalo semen of freezing. Experimental animals, housing, feeding schedule and the method of collection of semen are as per earlier paper (Bhosrekar, 1980). Freezing was carried out over liquid nitrogen vapour 4 cm above the liquid nitrogen level and was preserved at — 196°C under liquid nitrogen level.

Extracellular fluid after centrifugation of diluted semen (1:10 ratio) before and after freezing was soaked in 3 mm Whatman filter paper strips and dried at room temperature (15° to 30°C). The strips were fixed at one end of the gel prepared in petridish Acidic buffer system namely formic acid-acetic acid was used for running electrophorosis. The method of Aschaffenburg (1966) as used by Banerjee and Ganguli (1971) was followed for his study.

Similarly for studying polyacrylamide gel electrophorosis patterns method as adopted by Stephen (1973) was used.

For gel filtration pattern studies procedure adopted by Majumdar *et al* (1970) was used. Sephade xG-100 (pharmacia) was soaked for 120 hrs in distilled water. It was then freed of fine particles by decanting the supernatant. After the removal of fine particles the gel was equilibrated with buffer (Tris hydrochloric acid pH-8.0 and phosphate buffer pH 6.9). Gel was taken in LKB comumn 35×20 cm. 3 ml of the sample was layered, on the gel without disturbing the gel surface.

Elution was carried out in descending manner.

The effluents were continuously monitored by means of LKB Uvicord (Stephen, 1973) at 2537 um with chopper bar printing recording system.

The pure, Glycerol treated and frozen plasma of buffalo semen resolved into five components of which three were major. The CAWG (citricacid whey with Glycerol) before and after freezing and thawing showed similar trend but the bands resolved were faint because of dilution. Banerjee and Ganguli (1971) observed

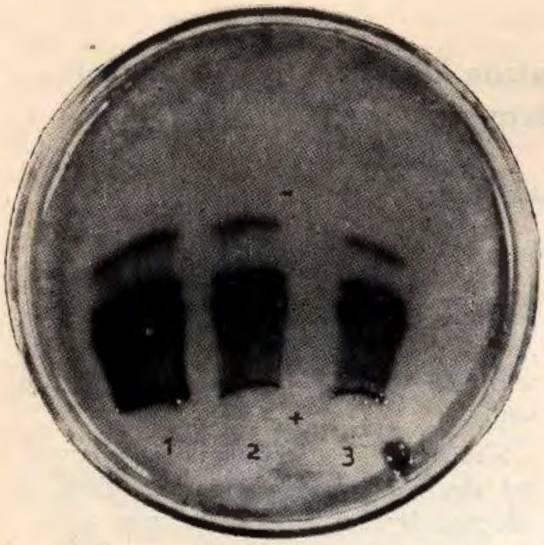


Fig. 1. Starch gel electrophorosis of buffalo Seminal plasma.
1. Pure seminal plasma.
2. Glycerol treated seminal plasma before freezing.
3. Glycerol treated seminal plasma after freezing.

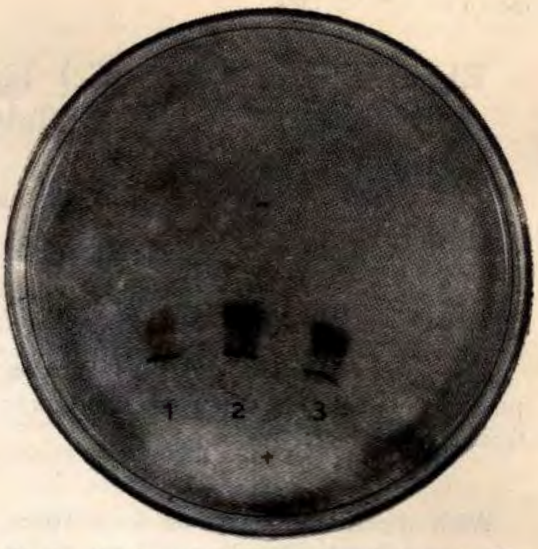


Fig. 2. Starch gel electrophorosis of citric acid whey (CAW) and diluted glycerolated buffalo semen before and after freezing.
1. Citric acis whey
2. Diluted glycerolated semen before freezing.
3. Diluted glycerolated semen after freezing.

similar pattern for buffalo semen, but reported two to be major, while Agar *et al* (1965) reported 8 fractions on paper electrophorosis with major three fractions. (Fig. 1 & 2).

Similarly the polyacrylamide gel using tris-citrate buffer system resolved undiluted buffalo semen in five distinct bands with 3 major, before and after freezing. (Fig. 3).

The diluted semen in CAWG and stored for different periods under liquid-nitrogen, also showed similar results. No differences were found in resolution and placement of bands except a reduction in concentration in diluted semen.

Similarly in molecular pattern as observed by gel filtration Technique no differences were recorded on freezing buffalo semen in (CAWG) citric acid whey with Glycerol diluent as compared to before freezing. In whey the second peak was observed to be shorter as compared to diluted buffalo semen. In undiluted seminal palsma of buffalo semen second peak was much higher than the first. The elution volume in all cases was approximately equal. Similar trend was recorded by using acidic buffer at pH 6.9. Banerjee and Ganguli (1971) also showed similar pattern for buffalo semen while CAW alone showed second peak to be shorter which was in accordance with Balakrishnan *et al* (1973) who reported gel filtration pattern for cow milk whey. (Fig. 4 & 5).

The author is thankful to Dr. N. C. Ganguli for his unstinted guidance while carrying out the work. He also wishes to thank Dr. D. Sunderasan the then Director NDRI for his keen interest in the studies.

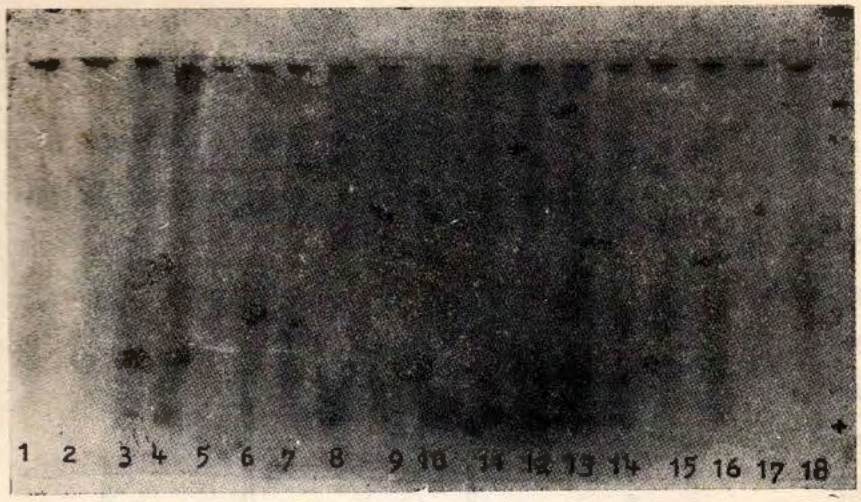


Fig. 3. Polyacrylamide gel electropharosis of buffalo semen before and after freezing using CAWG as a diluent at various intervals of storage under liquid nitrogen.

1. Frozen semen — 1 year old.
2. Frozen semen — 11 months old.
3. Frozen semen — 10 months old.
4. Frozen semen — 10 months old.
5. Frozen semen — 6 months old.
6. Frozen semen — 15 days old.
7. Frozen semen — 1 month old.
8. Frozen semen — 4 months old.
9. Frozen semen — 15 days old.
10. Frozen semen — 15 days old.
11. Whole semen — before freezing.
12. Whole semen — after freeeing.
13. Diluted glycerolated semen before feezing.
14. Frozen semen— 24 hours old.
15. Frozen semen — 24 hours old.
16. Diluted glycerolated semen before freezing.
17. Citric acid whey.
18. Diluted glycerolated semen before freezing.

Fig: 4

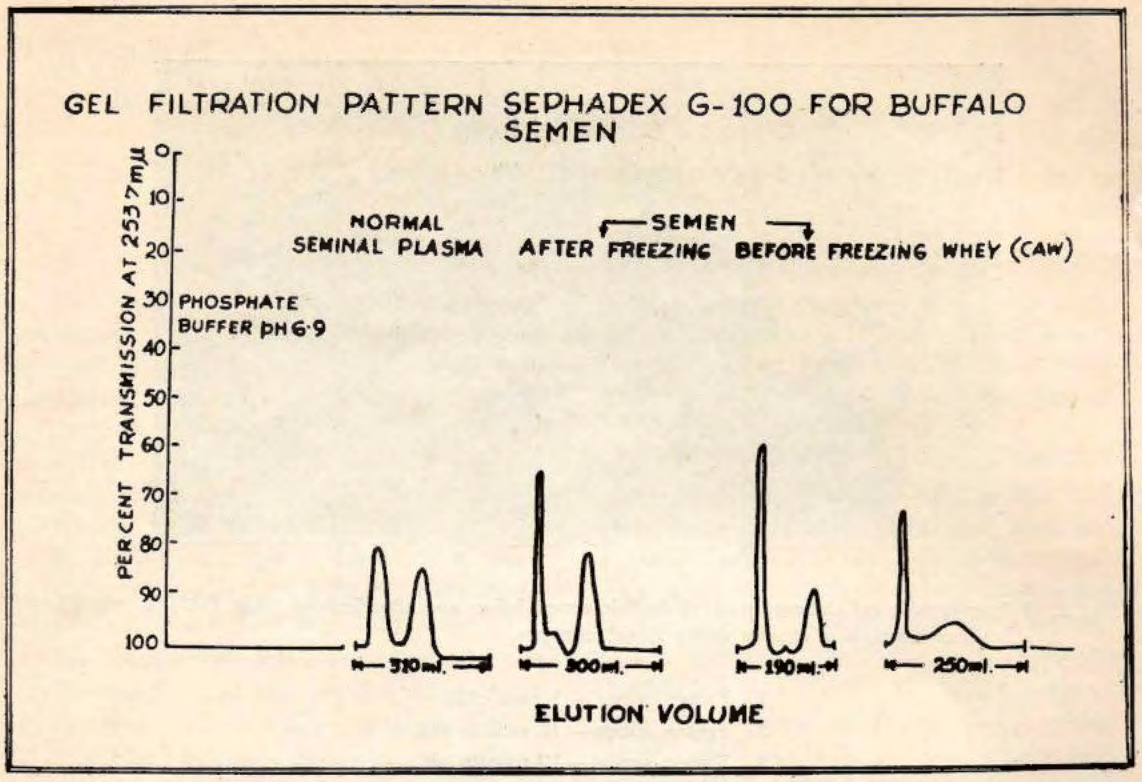
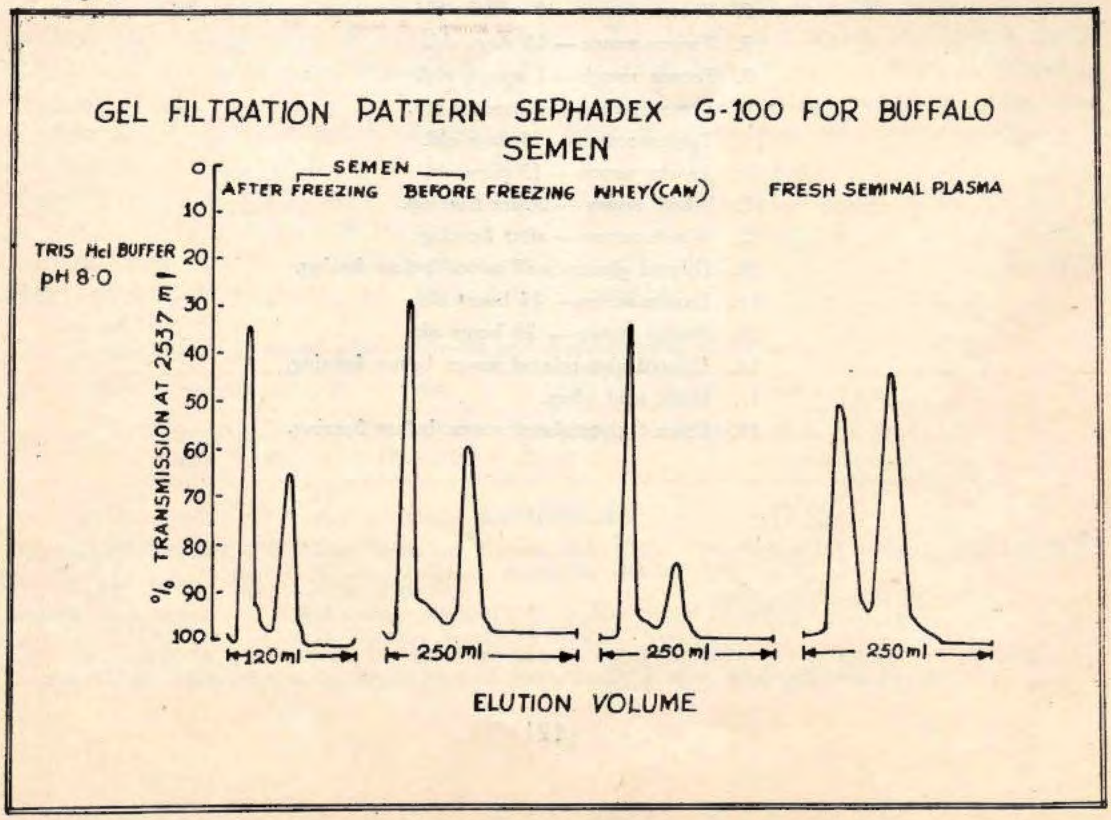

GEL FILTRATION PATTERN SEPHADEX G-100 FOR BUFFALO SEMEN
PERCENT TRANSMISSION AT 2537mμ
0
10
20
30
40
50
60
70
80
90
100
NORMAL SEMINAL PLASMA
SEMEN
AFTER FREEZING
BEFORE FREEZING
WHEY (CAW)
PHOSPHATE BUFFER pH 6·9
310ml.
300ml.
190ml.
250ml.
ELUTION VOLUME

Fig: 5


GEL FILTRATION PATTERN SEPHADEX G-100 FOR BUFFALO SEMEN
SEMEN
AFTER FREEZING
BEFORE FREEZING
WHEY(CAW)
FRESH SEMINAL PLASMA
TRIS Hcl BUFFER pH 8·0
% TRANSMISSION AT 2537 mμ
0
10
20
30
40
50
60
70
80
90
100
120ml
250 ml
250 ml
250 ml
ELUTION VOLUME

REFERENCES

Agar, N.S., Rawat, J.S. and Roy, A. (1965) Indian J. Dairy Sci. **18**: 101.

Balakrishnan R., Krishnaswami S.,and Vedanayakam, A.R. (1978) Cherion 2 (1): 31.

Banerjee, A.K. and Ganguli, N.C. (1971) J. Anim. Sci. **32**: 1212.

Bhosrekar M.R. (1980) Indian Vet. J. **57**, 806.

Bhosrekar, M and Ganguli, N.C. (1976) J. Reprod. fert. **48**: 215.

Ganguli, N.C., Bhosrekar, M. and Stephan, J. (1973) J. Reprod. fert. **35**: 355.

Majmudar G.C., Kumar, S. and Ganguli, N.C. (1970) Indian J. Biochem. **7**:(1): 60.

Stephen, J. (1973) Ph.D. thesis, Punjab University, Chandigarh.

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Changes In The Morphology Of Buck Spermatozoa Before And After Freezing

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ABSTRACT

The mean percentage of abnormalities of head, mid-piece, coiled tail and free normal head in different stages of processing and freezing did not differ significantly from that of neat semen in any extender.

* * *

Morphological changes of the spermatozoa in bull and ram frozen semen were studied by several investigators (Zakreewska, 1958; Semakov, 1975; Bania, 1978; Mollova, 1980). Perusal of available literature revealed no report on the effect of freezing on the morphology of buck spermatozoa. Hence, the present study has been undertaken to record the morphological abnormalities of buck spermatozoa during processing and freezing in different extenders.

Twenty ejaculates, 4 each from 5 indigenous bucks aged 3 to 5 years, collected twice weekly during June, 1983 using standard artificial vagina were used for this study. If the volume of first emjaculate was less than 0.8 ml, a second ejaculate was collected and pooled together. The semen was then diluted @1:5 with Tris buffer and centrifuged at 3000 rpm. The supernatant fluid was pipetted out. The sediment with little entrapped fluid was split into 4 parts and diluted @1:5 with fraction—A of egg yolk-citrate (Mathew, 1974), Tris (Flukiger *et al.*, 1976), skim milk (Rajkonwar *et al.*, 1977) and raffinose (Paggi, 1971) extenders. The dilution rate was based on semen volume prior to removal of seminal plasma. The initially diluted semen was cooled to 5°C within 1½ hour and then fraction-B of the extenders (volume equal to that of fraction-A) was added to it in 3 parts at an interval of 10 minutes. After 5 hours of equilibration at 5°3, the straws filled with semen (0.5 ml French Straw) were exposed to liquid nitrogen (LN2) vapour 5 cm above the LN2 level for 10 minutes and then stored in LN2. After 15 to 18 hours of storage, the semen was thawed in warm water (37°C) for 12 to 15 seconds.

One permanant eosin-nigrosin stained (Hancock, 1951) semen smear was prepared from neat semen immediately after collection, after initial dilution and cooling to 5°C, after equilibration, and after freezing and thawing in each extender. Thus, all total 260 smears were prepared, 13 each from 20 ejaculates. The incidence of various sperm abnormalities were estimated by counting 200 spermatocoa in each semen smear at a magnification of 1000x. The analysis of variance and critical difference test were conducted as per Snedecor and Cochran (1968).

In neat semen, the mean abnormalities

TABLE 1. The incidence (%) of bent tail (mean of 20 obsergations±S.E.) in neat semen and in different stages of processing and freezing in different extenders.

	Egg yolk-citrate	Tris	Skim milk	Raffinose
Neat Semen	0.20a±0.07	0.20a±0.07	0.20a±0.07	0.20a±0.07
After initial dilution & cooling	0.78ab±0.14	0.85ab±0.15	0.85ab±0.15	1.28b±0.24
After equilibration	1.05b±0.20	1.23b±0.19	1.05b0±.17	1.53b±0.30
After freezing and thawing	1.50b±0.19	1.26b±0.17	1.18b±0.16	1.54b±0.27

The means in each column bearing at least one superscript in common do not differ significantly

of head, mid-piece, coiled tail, bent tail and free normal head were found to be 0.20±0.08, 0.13±0.05, 0.28±0.09, 0.20± 0.07 and 0.63±0.10 per cent respectively. The mean percentage of abnormalities of head, mid-piece, coiled tail and free normal head in different stages of processing and freeeing did not differ significantly from that of neat semen in any extender. This is in agreement with that of Bonia (1978) and Mollova (1980).

The incidence of bent tails increased significantly ($P<0.01$) during processing and freezing in all the extenders. Critical difference test showed that the mean percentage of bent tails in neat semen, and after initial dilution and cooling to 5°C differed significantly ($P<0.01$) only in raffinose, but not in other extenders. The incidence of bent tail after initial dilution and cooling, after equilibration, and after freezing and thawing did not differ significantly in any extender (Table 1). There was no significant difference between extenders at any stage from cooling to freezing. Zakrzewska (1958) reported that the incidence of protoplasmic droplets, bent tails, tails or head stuck together decreased whereas the changes in the structure of head and mid-piece increased due to low temperature freezing. He, further, observed that the proportion of morphologically normal spermatozoa did not significantly change by freezing to −79°C in a diluent containing 20 per cent glycerol.

Acknowledgement

The authors are grateful to the Principal, College of Veterinary Science, A. P. Agricultural University, Tirupati for the facilities provided.

REFERENCES

Bonia, K.K. 1978. M.V.Sc. Thesis, Rohilkhand University, Bareilly, U.P.

Flukiger, A., Singh Sall, H. and Wieser, M.F. 1976. Deep freezing of buffalo bull semen using four different diluents. Proc. VIII Int. Congr. Anim. Reprod. & A.I., Cracow, Vol. **IV**: 798-801.

Hancock, J.L. 1951. A staining technique for the study of temperature shock in semen. Nature (London) **167**: 323-324.

Mathew, A. 1974. Principles and Practice of Deep freezing bull semen. Indo-Swiss Project, Kerala, India.

Mollova, M.V. 1980. Changes in the structure fram spermatoeoa after equilibration and deep freezing. Doklady Bolgarskoi Akademii Nauk, **33**: 393-395 (Fide Anim. Breed. Abstr. **50**: 1464).

Paggi, 1971. Artificial Insemination in goats. XIV.Experiments on deep freeeing of buck semen. Vet. Med. Dissertation, Tieraretl. Hochsch., Hanover, 51 PP (Fide Anim. Breed. Abstr. **39**: 4828).

Rajkonwar, C.K., Borgohain, B.N. and Sattar, A. 1977. The impact of A.I. and introduction of frozen semen in the development of cattle industry in Assam. VetCol. **17**: 39-44.

Semakov, V.G. 1975. Morphological changes in the bull sperm tail in semen diluents. Nauchno Issledovatel'skii Institut Sel' skogo Khoeyaistva Tsentral'nykh Raionov Nechernoeemnoi eony No. **30**: 133-136 (Fide Anim. Breed. Abstr. **43**: 2880).

Snedecor, G.W. and Cochran, W.G. 1968. Statistical Methods. 6th Edn., Indian edn. pp. 258-262, 302. Oxford & IBH Publishing Co., Calcutta.

Zakrzewska, G. 1958. The effect of cooling bull semen to — 79°C on the morphology of the spermatozoa. Plodnosc i neiplodnosc zwierzqt domowych. zesz. probl. prostep—Nauk roln. polsk Akad. Nauk, No. **11**: 165-170(Fide. Anim. Breed. Abstr. **26**: 1953).

IJAR 5: 1: 127 1984

A Note On Non-Utilization Of Endogenous Lipids And Phospholipids By Buffalo Spermatozoa

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Some reports indicate that washed spermatozoa utilize endogenous lipids more specifically phospholipids as an energy source when incubated in the absence of oxidizable sugars, fatty acids and amino acids. Since there was no report available on buffalo spermatozoa the present study was undertaken on this aspect.

Pooled semen samples from 5 Murrah buffalo bulls (approx. 5 years age) were distributed into 5 ml aliquots in tubes marked A, B, C. Tube A was kept as control. Tube B was centrifuged, seminal plasma removed and spermatoeoa were washed with calcium free Ringer phosphate buffer and finally spermatoeoa were suspended in total volume of 5 ml buffer. Tube C had whole semen. Tubes B & C were incubated at 37°C for 4 h after which spermatozoal pack was obtained by centrifugation. Total lipids and phospholipids were then estimated in spermatozoa in all the three tubes. For total lipids method of Folch *et al.* (1957) and for phosphorus method of Chen *et al.* (1956) was followed. This study was conducted ten times.

The results showed that there was no difference in both total lipids and phospholipids content of spermatozoa of whole semen and that of washed spermatozoa after incubation upto 4 h. period. Microscopic motility examination revealed that washed buffalo spermatozoa can maintain their motility to low level even upto 4 h. There is indication that washed buffalo spermatoeoa donot possibly utiliee these compounds even when exogenous substrates are not available. These results are in agreement to the reports of Scott and Dawson (1968) and Clegg and Foote (1973) who did not find any decrease in phospholipids after incubation of bull spermatozoa. The non-utilization of these compounds by whole semen is understandable as spermatozoa can use many exogenous substrates available from the seminal plasma to maintain their motility and activity, but how washed spermatozoa maintain their activity in the absence of exogenous substrates is really intriguing. There is possibility that buffalo spermatozoa utilize some other endogenous substrates to maintain their livability even upto 4 h during *in vitro* incubation.

REFERENCES

Chen, PS.S, Toribora, T.Y. (Jr.) and Warner, H. 1956. Micro-determination of phosphorus. Anal. Chem. **28**: 1756.

Clegg, E.D. and Foote, R.H. 1973. Phospholipid composition of bovine sperm fractions, seminal plasma and cytoplasmic droplets. J. Reprod. Fert. **34**: 379.

Folch, R.J., Lees, M. and Sloane-Stanley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226**: 497.

Scott, T.W. and Dawson, R.M.C. 1968. Metabolism of phospholipids by spermatozoa and seminal plasma Biochem. J. **108**: 457.

IJAR 5: 1: 128 1984

Blood Glucose Levels In Different Reproductive Status In Surti Buffaloes.

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The blood glucose level gives an indication of the carbohydrate status of an animal. The mean blood glucose concentration of the herd is valuable. All hypoglycaemic herds pose the problem of negative energy balance and suffer from anoestrum in particular during winter (King, 1971).

In the present report, the blood glucose levels were studied in different reproductive conditions in Surti buffaloes.

The blood samples were collected from 60 animals from the jugular vein after examining the animals for their reproductive status. Sodium flouride was added as an anticoagulant for the study of blood glucose. Modified titrimetric method of Asatoor and King for true sugar was employed as described by Wooten (1964).

The blood glucose levels found in different conditions in the animals under study were: 56.31 ± 6.23 mg% in animals in oestrus (15); 59.95 ± 9.05 mg% in animals with visible genital infections (14); 43.21 ± 6.74 mg% in anoestrus animals (18) and 62.58 ± 7.65 mg% in pregnant animals (13). The blood sugar levels in different reproductive conditions studied were found to be non-significant (Table-1).

However, on application of Duncan's New Multiple Range Test (Pillai and Sinha, 1968), it was observed that the levels of blood glucose in anoestrus animals were significantly lower than those of animals in oestrus, animals having visible genital infections and pregnant animals. These results are in agreement with the findings of King (1971), Patil (1976), Mokashi *et al* (1974), Dhoble and Gupta (1979) and Chauhan *et al* (1981) studied in cows and buffaloes.

The levels of blood glucose were significantly higher in animals in oestrus, animals with visible genital infections and pregnant animals when compared with the levels in anoestrus animals. It is apparent from the results reported above that for the regular cyclicity, optimal blood glucose levels are considered valuable.

TABLE 1 Blood glucose levels znd Reproductive conditions.

Ssurce	D.F.	S.S.	M.S.	Cal. F.	Table F value
Between phases	3	3538.9001	1179.6333	1.4299NS	2.78 at 5% level
Within phases	54	44546.4511	824.9342		of significance

REFERENCES

Chauhan, F.S. Takkaer, O.P., Maher Singh and Tiwana, M.S. (1981) The Indian HJ. of Anim. Repro. **1**: 31-36.
Dhoble, R.L. and Gupta, S.K. (1979) Indian J. Anim. Sci. **49**: 953-954.
King, J.O.L. (1971). Vet. Rec. **89**: 320-324.
Mokashi, B.G., Deshpande, R.B. and Sane, C.R. (1974) Indian Vet. J. **51**: 671-675.
Patil, J.S. (1976). M.V.Sc. thesis, Bombay Veterinary College, Bombay.
Pillai, S.K. and Sinha, H.C. (1968) Statistical Methods for Biological Workers. Ramprasad and Sons, Agra.
Wooten, I.D.R. (1964) Micro-analysis in Medical Biochemistry. J. & A. Churchill Ltd., London.

IJAR 5: 1: 129 1984

'Nocardia Pelletieri Isolated From The Uterine Discharge Of An Aborted Cow.

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Mycotic abortion due to *Nocardia asteroides* has been reported in bovines (Ainsworth and Austwick, 1973). No other species of genus Nocardia are known to cause abortion in cattle. *Nocardia pelletieri* is a human pathogen, causes subcutaneous and mycetomal infections in man and has not been reported from animals so far (Jungerman and Schwartzman, 1972).

An aseptically collected uterine discharge from a cow having third trimester abortion, was sent to the Dept. of Bacteriology, Bombay Veterinary College for bacteriological isolation. The material was found negative for Brucella species and anaerobic microflora. The aerobic isolate was a coagulate negative and nonhemolytic staphylococci.

The bacteriological finding not being significant, the material was subjected to mycological investigation. A five day incubation at room temperature revealed orange red coloured colonies on Sabouraud's dextrose agar. The colony morphology was glabrous to raised and wrinkled with soft consistency. The organism grew with similar colony characters with no hemolysis on blood agar at 37°C after three days. The culture isolate was stained by Gram's method and microscopic view of the organism was Gram positive bacillary and coccoid forms with mycelial elements stained unevenly giving a beaded appearance. The organism rendered non-acidfastness by cold Kinyoun acidfast technique using 1% H_2SO_4 (aquous) for decolorization. The isolate was identified as *Nocardia pelletieri* on the basis of biochemical tests as described by Jungerman and Schwartzman (1972).

It is difficult to assess the pathogenic role of *Nocardia pelleteiri* in bovine abortion from a single case. However earlier reports on mycotic abortion due to *Nocardia asteriodes* (Ainsworth and Austwick, loc. cit.) signify the present finding to an extent and necessitate further research work.

REFERENCES

Ainsworth, G.C. and Austwick, P.K.C. (1973). Fungal diseases of animals 2nd Ed., p. 76, Common Wealth Agricultural Bureaux. Slough, England.

Jungerman, P.F. and Schwartzeman, R.W. (1972). Veterinary Medical Mycology, Lea and Febiger, Philadelphia.

IJAR 5: 1: 130 1984

Leakage Of Hyaluronidase Enzyme and Deep Freezing Of Semen

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The hyaluronidase enzyme which is solely of testicular origin has been considered the best "marker enzyme" for the acrosomal integrity. Hyaluronidase activity in sperm extract fluid has been found to be significantly related with fertility. (Raizada et al, 1980).

The role of seminal hyaluronidase in fertilization process consists in denuding the egg of its surrounding follicular cells by acting on hyaluronic acid, so as to enable the spermatozoa for penetration and fertilization. Hyaluronidase enzyme was estimated according to Bollet et al (1953).

In the present experiment, the significant difference in hyaluronidase activity was found between diluted huffalo semen samples before freezing and freeze-thawed semen samples. The enzyme activity obtained was expressed as difference in optical density (O.D.) readings read from spectro-photocolorimeter. It averaged 0.064 and 0.065 O.D. for Tris Fructose Yolk Glycerol and Lactose Fructose Yolk Glycerol diluted semen samples before freezing which decreased to 0.039 and 0.030 for both the dilutors respectively in freeee-thawed semen samples.

The hyaluronidase enzyme activity in sperm extract of buffalo semen significantly ($P<0.05$) decreased after freezing and thawing. The difference in hyaluronidase enzyme activity between the dilutors under study was found to be non-significant.

REFERENCES

Bollet, A.J.; Bonner, W.M.Jr., and Nance, J.L. (1953) J. Biol. Chem. 238: 3522.

Raizada, S., Singh, M and Chaudhary, K.C. (1980) J. Agri. Sci. *90*: 487-488.

IJAR 5: 1: 131-132 1984

Bovine Infertility–A Potent Hazzard For Genetic Improvement In Milk Production

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The galloping growth rate of human population demands a rapid improvement in milk production. Genetic engineering to cause a drastic break through in White Revolution has its own limitations. The important handicap is low heritabiilty of milk production. Bovine infertility being a major land mark in the path of genetic progress has all the more very little scope of mass selection. The additive variability is almost negligible in so far as the bovine fertility is concerned. The major consequence of such situation is the prolonged generation gap which severely cuts down the annual rate of genetic progress. This can be very easily seen from the following equation $\Delta G = \frac{h^2 \times SD}{G.I.}$ where the annual genetic progress (ΔG) is the function of heritability (h^2), selection differential (SD) and the generation interval (GI). Selection Differential is the difference between the average of selected parents and the herd average and the Generation Interval is the average age of individuals when they become parents.

Efficient reproductive fitness minimises the generation interval and thereby improves the annual genetic gain. Reproductive efficiency is a function of fertility which has proportionately negligible genetic variability in relation to the non-genetic variation. Bovine infertility therefore calls forth a critical review of the factors that are responsible for such condition. In a broad sense man, bull and the cow are the most important contributing members for this consequence. In the published literature, although the reproductive efficiency has been shown to be lowly (0.04) heritable, yet it is said to have positive phenotypic (0.05) and genetic (0.38) correlations with the lactation yield (Sharma 1983). This clearly indicates the possibility of higher lactation yield through selection of parents having better reproductive efficiency.

The factors that are responsible for inducing the bovine infertility have been extensively studied in India and abroad. The word infertile bears specific meaning for each of its letters viz. Inherited defects, Nutritional deficiency, Failure of growth and Development, Resistance loss, Termination of pregnancy, Impotancy, Lack of skill and interest and Embryonic death. These studies have concluded that reduced ovarian activity, persistant corpus luteum, cystic ovarian degeneration, genetic and the congenital mal-formation and the pathological conditions of genetalia are the main causes. Seasonal advantages in ovarian activity have been reported by the several workers (Gurdial Singh *et al* 1983) during October and November months. Madhu *et al* (1983) had indicated that infertility in

cross-bred cattle due to anestrus, under developed genital organs, repeat breeding, persistant corpusleuteum, cystic ovarian de-generation and other functioning disorders were 24.20, 18.10, 17.97, 3.25, 2.84 and 0.66% respectively. Sharma et al (1983) had reported the indidence of repeat breeders has 27.1, 18.8 and 11.2% in F×H, B×H and J×H cross-breds, respectively. They had also observed an increase in incidence of repeat breeders after 4th lactation. Nowshahri *et al* (1983) had observed that almost 2/3 of such females when treated with 7 mg of dithylstillbestrol per 100 kg body weight can be cured. This is quite useful to preserve some of the superior genetic material provided these are non-hereditary in nature. Genetic Improvement and clinical restoration of fertility do not go side by side because in making genetic progress, the carriers of un-desirable genes must go out from the breeding population to reduce the frequency of such genes. Fortunately many of such characters are governed by recessive genes with incomplete penetrance and hence Culling is practically easier.

The factors which induce infertility are the real victims of stress. Better management, feeding, breeding and weeding certainly take care of them and prepare the individuals to become stress resistant. In the absence of which a vicious circle starts and the genetic potential is never expressed properly. Since the level of nutrition, management practices, seasons and pathological conditions significantly affect the bovine fertility, these must be perfect to enable the cattle breeders to select the right type of parents. Further, since the selection opportunities are limited for reproductive efficiency, improved managerial practices as discussed above are very essential to harvest maximum benefits of hybrid vigor through cross-breeding.

Acknowledgement

The Author is highly grateful to the Indian Society for the study of Animal Reproduction for extending invitation to present this paper at the Gujarat Chamber of Commerce, Ahmedabad on 13th May, 1984.

REFERENCES

Sharma, O.P. (1983) Appraisal of Breeding efficiency and its association with Milk Production in Buffaloes. IJAR, 3: 1-93.

Nowshahri, M.A. and Maurya, S.N. (1983) Reproductive Performance of Infertile Cattle induced to lactation. IJAR, 3: 1-93.

Sharma, N.C., Luktuke, S.N. and Gupta S.K. (1983) Incidence of Repeat breeding in Cross bred cows. IJAR 3: 1-110.

Gurdial Singh, Singh, G.B. and Sharma, S.D. (1983) Studies on initiation of ovarian activity following summer season in village buffaloes. IJAR 3: 1-23.

IJAR 5: 1: 133-134 1984

THESIS

ABSTRACTS

Studies On Certain Aspects Of Gynaecology In Kankrej Cattle And Its Exotic Crosses. M.V. Sc. Thesis

Student: NM PATEL Guide: DR SN LUKTUKE

The present study was undertaken with the object of knowing the reproductive performance pertaining to age at first calving, interval between parturition and post-partum first and fertile heat and intercalving period of Kankrej and its exotic crossbreds (Kankrej × Jersey and Kankrej × Holstein-Friesian). In addition, the incidences of reproductive disorders viz., anoestrum, repeat breeding and pregnancy losses prevalent in the farms under study were assessed by periodical sexual health control programme during the period of study (March 1982 to February, 1983). The present study was carried out at the Cattle Breeding Farm, Mandvi, Livestock Research Station, Dantiwada and the Dairy Herd of Shri Surat Panjarapole, Surat on 504 breedable females including 53 Kankrej heifers, 17 Kankrej crossbred heifers, 413 Kankrej cows and 21 Kankrej crossbred cows.

The age at first calving in Kankrej heifers was 2101.72±58.19 and 1282.37±29.20 days, in K × J heifers it was 862.33 ±37.50 and 1031.00±0.00 days and in K × HF heifers it was 909.33±139.23 and 894.76±41.12 days at C.B.F., Mandvi and L.R.S., Dantiwada respectively. The difference in age at first calving was found to be significant ($P<0.01$).

The interval between parturition and first heat & fertile heat in Kankrej cows was 145.47±9.20 & 257.01±13.74, 92.54±8.27 & 177.14 and 195.26±9.23 & 313.57±12.08 days at C.B.F., Mandvi; L.R.S., Dantiwada and S.P.P., Surat respectively. Post-partum first & fertile heat intervals in K × J cows were 76.83± 13.13 & 134.00±37.71 and 94.66±50.11 & 43.00±0.00 days at C.B.F., Mandvi and L.R.S., Dantiwada respectively. In K × HF cows these postpartum intervals were 94.66±07.41 & 278.75±66.02 and 181.00±53.69 & 122.00±44.00 days at C.B.F., Mandvi and L.R.S., Dantiwada respectively. The differences in interval between parturition and first and fertile heat of Kankrej cows observed at 3 farms were found to be varying significantly ($P<0.01$).

The intercalving period in Kankrej cows was 542.40±14.97, 432.25±10.33 and 551.00±18.72 days at C.B.F., Mandvi, L.R.S., Dantiwada and S.P.P., Surat respectively. The intercalving period of K × J & K × HF cows were 426.5±40.5 & 484.5±14.5 and 395.23+32.17 & 420.33+34.26 days at C.B.F., Mandvi and L.R.S., Dantiwada respectively. The differences in intercalving period of Kankrej cows observed at 3 farms were found to be significant ($P<0.01$).

From the study on paritywise inter-

calving periods of farm born Kankrej cows at C.B.F., Mandvi it was observed that intercalving period decreased upto 3rd lactation and again started increasing from the 4th lactation onwards.

At the close of this study, in Kankrej heifers the incidences of true anoestrum were 25.00 and 13.15 per cent and cyclical C.L. was found in 50.0 and 18.3 per cent of reported anoestrous heifers at C.B.F., Mandvi and L.R.S., Dantiwada respectively. In Kankrej cows incidences of true anoestrum were 20.83, 3.28 and 5.61 per cent and cyclical corpus luteum was found in 8.33, 4.91 and 11.23 per cent of reported anoestrous cows at C.B.F., Mandvi; L.R.S., Dantiwada and S.P.P., Surat respectively. The incidences of repeat breeding in Kankrej heifers were 6.25 and 15.78 per cent at C.B.F., Mandvi and L.R.S., Dantiwada. These incidence in Kankrej cows were 19.04, 8.19 and 11.79 pre cent at C.B.F., Mandvi; L.R.S., Dantiwada and S.P.P., Surat respectively. The incidences of cystic ovarian degeneration in Kankrej heifers were 2.63 per cent at L.R.S., Dantiwada and in Kankrej cows the incidences were 1.58, 0.81 and 0.56 per cent at C.B.F., Mandvi, L.R.S., Dantiwada and S.P.P., Surat respectively. As regards pregnancy losses, incidences of abortions were only recorded in the farms under study. During the period of study no abortions have been recorded in heifers and cows at C.B.F., Mandvi while 2.48 and 1.88 per cent abortions were recorded in Kankrej cows at L.R.S., Dantiwada and S.P.P., Surat respectively.

Blood haemoglobin and serum total proteins values in 15 pregnant, 21 normally cycling, 16 anoestrous Kankrej cows were 14.07 ± 0.35 and 8.38 ± 0.46, 14.86 ± 0.28 and 8.11 ± 0.30, 13.45 ± 0.38 and 8.51 ± 0.38 gm% respectively. The differences in blood haemoglobin values in 3 reproductive groups were found to be differing significantly ($P<0.05$) while for serum total proteins the differences were non-significant.

IJAR 5: 1: 135-136 1984

Gynaecological, Microbiological And Histopathological Investigations With Therapeutical Considerations In Repeat Breeder Bovines. Ph. D Thesis

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The present study was undertaken with objectives to elucidate some of the aetiological factors involved in repeat breeding conditions and suggest therapeutical measures.

Gynaecological examination, of a total 318 repeat breeding buffaloes and 81 repeat breeding cows showed 40.67 and 49.15 per cent genital disorders respectively.

Inter-oestrual lengths of 1121 oestrous cycles in 243 repeat breeding buffaloes and 584 oestrous cycles in 81 repeat breeding cows were studied. Abnormal cycle lengths were 63.25 per cent in buffaloes and 54.46 per cent in cows.

Occurence of oestruses in repeat breeding buffaloes during different months of the year were studied. Out of 1698 oestrous periods studied, 1101 (64.80%) were exhibited during high breeding season (September-February) and 597 (35·16%) during low breeding season (March-August).

A total of 24 chronic repeat breeder cows were further investigated for histopathological changes in the genital tract. The major pathological conditions were chronic cervicitis, acute or chronic inflammatory changes in the endometrium, salpingitis and cystic ovarian degeneration.

Cervico-vaginal mucous samples from 117 repeat breeder animals were investigated for isolation of bacteria. Out of them 98 (83.76%) samples were found positive. The antibiotic sensitivity test revealed wide variation in the sensitivity pattern. The majority of the samples were resistant to Penicillin and Sensitive to Chloramphenicol and Gentamycin.

Ovulatory disturbances were studied in 141 repeat breeding buffaloes. The extent of delayed and anovulatory conditions were 16.56 and 15.09 per cent respectively.

Tubal patency test was done in a total of 130 repeat breeder animals by air insufflation apparatus. Out of them 37 (28.46%) animals showed unilateral and 22 (16.92%) bilateral blockages.

In 26 repeat breeder cows PSP test was performed for testing the tubal patency. In these cows, the unilateral and bilateral tubal blockages were 38.46 and 7.69 per cent respectively. The test proved highly accurate.

After diagnosing the aetiological factors, repeat breeder animals were treated accordingly. For infectious causes different antibiotics were used as per their sensitivity. The pregnancy rate was 68.70

per cent. For the treatment of ovulatory disturbances, ovulatory drugs were used, resulting into 66.66 per cent pregnancy. While in all 130 repeat breeder animals which were tested for fallopian tube patency, antibiotic solution was infused into the uterus under pressure. Among the followed 56.36 per cent animals became pregnant.

IJAR 5: 1: 137-138 1984

Gonadal Development And Activity In (Surati) Male Buffalo Calves From Birth To Sexual Maturity

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A THESIS SUBMITTED TO GUJARAT AGRICULTURAL UNIVERSITY FOR M.V.Sc. DEGREE IN REPRODUCTIVE BIOLOGY, YEAR-1983.

Gonadal development and activity in *Surati buffalo calves was studied from* birth to maturity (in 17 stages), to correlate the gonadal histology with testicular and serum biochemical characteristics. The characteristics studied,histologically in H. and E. stained sections included, seminiferous tubule diameter, tubular count, interstitial space per cent and various cellular elements in seminiferous tubule and the leydig cells; the biochemical estimates were Alkaline Phosphatase (AKP), protein, cholesterol (free and total), calcium and phosphorus, both in testis and serum: the nucleic acids were estimated in gonadal tissues only. Similar studied were also made in calves, hemi-orchidectomised at birth from Day 1-300.

Seminiferous tubule diameter increased four fold during the period of study. The tubular count and interstitial space decreased during the same period. The cellular components at birth were only gonocytes and basal indifferent cells. The appearence of primary spermatogonia, primary spermatocyte, round spermatid, elongated spermat d, and spermatozoa was observed at 200th, 330th, 450th, 480th and 510th days respectively. Sertoli cells were seen right from birth and the *Leydig cells started transforming from* Day 125 onwards long with lumen formation. Both the Leydig cell transformation and lumen formation was completed by 420th day of age. Serum and testicular AKP were high at birth to 28 days and showed a gradual declining pattern as the age increased, but typically the testicular AKP underwent a second peak at 42*n* to 450 days of age, while it was declining in the blood serum. Serum protein and testicular protein and nucleic acids also followed a similar pattern, also the cholesterol in serum and testis. But in serum a second rise of cholesterol concentration occurred during 420-450 days of age. The free cholesterol percentage was very low (about 15 per cent) in serum, whereas it was very high (30-60 per cent) in testis. The calcium and calcium to phosphorus ratio in serum were high soon after birth, but declined later to stabilize. The serum phosphorus level, at birth, were low and increased thereafter. The calcium and phosphorus content in gonadal tissue was very low and the ca:P ratio never rose above one as compared to serum where the ratio

was always above one. The various characteristics studied indicated the changes at puberty and sexual maturity. First month of life, then 4th to 7th month as well as 14 to 15 months of age showed critical developmental changes and activity in both chemical estimates and functional anatomy. It is clear from the present study that the buffalo calves sexually matured at an earlier age and can be used for breeding at a younger age than the one believed. It also implicates that proper management effects positively the age at sexual maturity and helps to bring it down.

Total Number of pages in the thesis-288

Number of words in Abstract-350

FARM NEWS

Feeding schedule for calves:

1st to 4th day.

Colostrum feeding @ 12% of body weight in a day, in divided 2 to 4 feedings a day.

5th to 60th day—

Whole milk feeding @ 10% of body weight in a day. In two feedings in a day.

61st to 90th day—

Whole milk feeding 3 kg in a day. In two feedings. If facilities permit, feed reconstituted milk replacer or skim milk instead of whole milk.

At the second week of age introduce calf-starter or conventional concentrates and excellent quality roughages. Feed increasing quantity of calf-starter and roughages. A healthy calf should consume calf-starter at the rates mentioned below at the indicated age.

1st month — 100 gms.
2nd month — 200 gms.
3rd month — 350 gms.
4th month — 500 gms.
5th month — 500 gms.
6th month — 500 gms.

INFORMATION ON VACCINATION OF CATTLE

Sr. No.	Disease	Type of Vaccine	Age of Vaccination	Route	Dose	Immunity	When to Vaccinate Season of Vaccination	Remarks
1.	Anthrax	Anthrax spore vaccine (IVRI), Izatnagar.	Above 6 months of Age	S/C	1 ml.	1 year	About 20 days before onset of monsoon	Immunity develops in about 10 days
2.	Haemorrhagic Septicaemia	H.S. oil adjuvant Vaccine for clean vaccination (IVRI), Izatnagar	,,	I/M	2 ml for animals weighing upto 140 kg & 3 ml for Animal weighing above 140 kg.	1 year	— Do —	Immunity develops in about 21 days
3.	— Do —	H.S. alum precipitated vaccine for vaccination especially during outbreak	,,	S/C	5 ml for animals weighing upto 500 lbs. & 10 ml for animals weighing above 500 lbs.	5 months	During actual outbreak	Immunity develops in about 10 days
4.	Black Quarter	Black quzarter Vaccine	,,	S/C	5 ml & 10 ml as above.	1 year	Preferably in May/ June. In endemic area in all the seasons	Second vaccination 10 days after the 1st inoculation where high degree of immunity is considered essential
5.	Foot & Mouth	FMD Vaccine of Hoechst or Raksha (NDDB) vaccine	As per company's instructions, schedule is to be followed.		—	—	—	To be kept on ice until it is vaccinated
6.	Rinderpest	R.P. Tissue culture vaccine.	6 months and above	S/C/	1 ml	3 years	—	—do— plus it should be used within 2 hrs after reconstitution
7.	Brucella	Brucella Cotton Strain 19 vaccine	4-8 months of age	S/C/	5 ml		—	—
8.	Rabies	Post-bite vaccines— (a) Semple's Vaccine (b) Bio-med Vaccine	Any age group	—	—	When the case is reported, start vaccination as early as possible		— Do —

IJAR 1: 4: 141-143 1984

ISSAR NEWS

1. AN APPEAL

The Indian Society For The Study Of Animal Reproduction Nils Lagerlof Memorial Fund

Veterinarians and livestock breeders in almost all the countries in the world are fully aware of the outstanding contribution made by late Prof. Nils Lagerlof in the field of Animal Reproduction. The Veterinary profession in India in particular will always remember him for his substantial assistance and advice in elevating the standard of veterinary education and research on par with the other countries of the world.

Late Professor Lagerlof was Head of the Department of Obstetrics and Gynaecology and Dean, Royal Veterinary College, Stockholm, Sweden (1948-1957; 1957-1962). On his retirement in the year 1962 he was Honoured as Professor Emeritus. He did very useful research in the field of Animal Reproduction and his contributions on Gonadal Hypoplasia, Sperm Morphology, Hereditory forms of infertility and sterility, Sexual Behaviour in the Bovines, Vibrio investigations and Biological aspects of infertility are praise worthy. He devoted his life mainly to teaching and research until 1950, but thereafter he was invited by Governments of various countries to offer his expertise advice on problems concerning Veterinary Education in the field of physio-pathology of Reproduction, Animal Breeding and Artificial Insemination.

He rendered very valuable technical advice on sexual health control and on infertility problems and breeding by artificial insemination to the Governments of Israel, India, Thailand, Turkey, Egypt, Pakistan and some of the African and Latin American countries. He was the member of the F.A.O. Expert Panel on Livestock Infertility (1954-62); President of the International Standing Committee on Animal Production (1948-61); President of the Scandinavian Veterinary Association on Reproductive Physiology and Pathology (1951-70); Member of the Swedish Agricultural Research Council (1945-57); Member of the Swedish Academic of the Agriculture; Member of the Advisory Scientific Committee to the Swedish Veterinary Board (1937-64); Member of the Scientific Council of the Swedish Dairy Association.

He was felicitated by the awards of Honourary Doctorate Degrees which were confirmed on him by the University of Stockholm, Hanover, Helsinki and London; and membership of the American Society for the study of Sterility; and corresponding Member of the Society from Madrid, Finland, Norway, Japan and various other associations in Sweden.

In appreciation of his meritorious services he was decorated as Knight Commander of the First Class of the Royal Order of the North Star and

Commander Order of the Republica Italiana, Finland, Norway and Knight of the First Grade of the order of the Danish Danebrog.

In India, with the advent of launching of various schemes and projects for cattle development, a number of problems cropped up with regard to fertility and infertility and Government of India, therefore, requested the F.A.O. to secure the services of Professor Nils Lagerlof an eminent Scientist in the field of Animal Reproduction. His services were assigned to India and he visited this country first in 1953 and after surveying the position made valuable suggestions to the Government of India in respect of Veterinary Education, Research and Extension. As per his recommendations almost all the Universities in India have prescribed the uniform syllabus for the subject of Obstetrics, Gynaecology, Andrology and Artificial Insemination in the Veterinary curriculum and new departments for the subject have been established in each of the Veterinary College. This has given an impetus to research in Physio-Pathology of Reproduction and very valuable findings have been placed on record. Due to his wider outlook and zeal to spread education and research he undertook the responsibility of educating veterinary personnel from India and other countries of the world by organising special type of courses every alternate year in Sweden. About 45 members of the teaching staff from different Universities in India got the benefit of such advanced training courses in Sweden which enabled them to conduct higher research. Prof. Lagerlof visited India every alternate year till 1969 and every time by reviewing the position he made vital suggestions to the Government of India. He established International co-operation in veterinary education through which a net work of Scientists was spread throughout the world for conducting research in Animal Reproduction. Credit goes to him for his vital interest in the Indian Veterinary Profession. It is due to his influence that a lot of substantial aid has come to a number of Veterinary Colleges in India by way of equipments, books etc.

It may be said without hesitation that he was the only professor who not only trained Veterinary Scientists for higher research but who followed them year after year to underst and the research work they have on hand and help them not only by advice but by extending all possible technical aid by way of precision equipment, literature etc. He was regularly mailing the most uptodate research publications to almost all the candidates who had undergone training in Sweden.

Prof. Lagerlof was a Scientist and an educationist of an outstanding competence and foresight. He had a remarkable ability of stimulating enthusiasm in young research workers by directing their attention to the heart of a problem To Governments of many countries and Veterinarians he will be remembered as a Vice Councillor whose advice on Livestock Production has laid to the establishment of sound policies in India. By his dynamic and genial personality he has established a network of sicentists all over the world.

In recognisition of his yeomen services to Indain Veterinary profession, the delegates attending the F.A.O./SIDA Follow up Seminary on Animal Reproduction held at Bangalore in November, 1972 unanimously decided to raise "Nils Lagerlof Memorial Fund." As a mark of profound respect it was decided to institute a Gold Medal in his Memory to be

awarded to an outstanding publication in the field of Animal Reproduction every year. There was an appreciable response and all the delegates attending the Seminar contributed a sum of Rs. 100/- each including Dr. Settergren, Director of the Seminar. An amount of over Rs. 3,000/- was collected on the spot at the very first meeting of the foundation members. The Memorial fund would be operated through the Indian Society for the Study of Animal Reproduction. Considering the golden objective the President and the Members of the Executive Committee earnestly appeal to one and all for generous contribution to rasise the fund to substantial figures.

Donations may be made in the name of "The Indian Society for the Study of Animal Reproduction", the cheques/cash money orders may be sent to the Treasurer of the Society.

DR. C. R. SANE
Patron, ISSAR

2. Honours

"ISSAR takes great pleasure to learn that one of our Honoured Life Member, Dr. G. B. Singh, Professor Emeritus Punjab Agricultural University, Ludhiana, has been conferred the Degree of Science (Honoris Causa) by the Orissa University of Agriculture and Technology at its convocation held on 4th March, 1984. Probably this is the first time that an Indian Veterinarian has been thus honoured by an Agricultural University. Congratulations.

ISSAR thanks profusely the Orissa University of Agril. & Tech. for recognising the meritorious work done by the Veterinarian of repute Dr. G. B. Singh. It is hoped that other Agricultural Universities will follow this example of honouring eminent veterinarians"

3. Notification

3. Nils Lagerlof Memorial Award-1983

The Indian Society for the Study of Animal Reproduction is pleased to invite Research/Clinical articles on the subject of Animal Reproduction published by the Indian Authors in any of the journals during Jan. to Dec. 1983, for consideration of the Nils Lagerlof Memorial Award for the year 1983.

Four copies of the reprints of the article should be sent by the author to the Hon. Secretary ISSAR (C/o. Dept. of Animal Reproduction, Bombay Veterinary College, Parel, Bombay-400 012). The articles should reach the Secretary ISSAR latest by 30th Nov. 1984. The Award will be presented at a suitable function of ISSAR.

DR. D. P. VELHANKAR
Hon. Secretary, ISSAR

4. Our Talent

Dr. Man Mohan Singh, Research Associate JNKVV, Jabalpur has the unique honour to come out with flying colours in the Indian Administrative Services Examinations (Group A) conducted by U.P.S.C. in 1983. He has a brilliant academic career. JNKVV awarded certificate of honour with the degree of B.V.Sc. & A. H. for his overall excellent academic record. He is also the recipient of Junior and Senior research Fellowship of I.C.A.R. He did his M.V.Sc. & A. H. in the subject of Obstetrics and Gynaecology securing 4.00/4.00 OCGA. He has won many a cups and trophies in the annual sports of the college.

Dr. M. M. Singh is an ISSAR member and ISSAR appreciates his coveted achievements and wish him all the success. It is hoped that in his I.A.S. career, he would also remember his duty towards the animal world.

5. ISSAR Gujarat Chapter

a.

A seminar on 'Bovine Infertility Problems was held on 13th May '84 at Gujarat Chamber of Commerce, Ahmedabad. The inauguration of the seminar was done by Dr. C. R. Sane, Patron ISSAR. Dr. T. N. Vaishnav, Director of Animal Husbandry, Gujarat State was the chief guest for the occasion. Dr. L. G. Patel, Cattle Development officer gave a ceremonial speech. And Dr. B. R. Deshpande, President, ISSAR gave a presidential speech.

In the technical session the following papers were read and discussed.

1. Bovine infertility — a hazard for genetic improvement in milk production. Dr. A. P. Vyas
2. Studies on True Anoestrous condition in Surti buffaloes and trials with Prajana. Dr. I. M. Shah
3. Bovine Anoestrum Prof. Dr. S. B. Kodagali
4. Role of frozen semen technology in improving fertility Dr. Chakraborthy
5. Repeat breeding in cattle Dr. F. S. Kavani
6. Cyclic pattern in repeat breeders Dr. H. J. Derashri
7. Some Practical Suggestions in Animal Reproduction. Dr. B. K. Bhavsar

About 200 delegates attended this. Seminar. The seminar was a great success.

b.

Indian Society for the study of Animal Reproduction, ISSAR, Gujarat Chapter, General body meeting was held on 13-5-'84 at Ahmedabad. The office bearers for the period of three years were unanimously decided.

President: Prof. Dr. S. B. Kodagali
Vice-president: Dr. B. M. Bhatt
Secretary: Dr. I. M. Shah
Jt. Secretary: Dr. F. S. Kavani
Treasurer: Dr. H. J. Derashri
Committee members:
Dr. S. H. Dhulkhed
Dr. B. K. Bhavsar
Dr. S. H. Parekh
Dr. D. B. Gorani
Dr. V. R. Jani

6. Recommendations made at the plenary session of the 5th All India National Congress on Animal Reproduction held at Pant Nagar, Dist. Nainital (U. P.) from 27-2-84 to 29-2-84.

The delegates present at the concluding plenary session of the National Congress on Animal Reproduction unanimously felt and resolved to submit the following recommendations that:

(1) There should be greater co-ordination among the research workers, field veterinarians and the Industry. The research laboratories should get the feed back from the field and the Industry should render financial assistance to overcome the problems pertaining to reproduction and production.

(2) The cross bred bulls semen being of poor quality, multipronged investigations in the field of breeding, Nutrition etc. need be carried out before introducing the bulls in breeding programmes and further recommended that periodical check ups at regular intervals of such bulls for Andrological soundness be scrupolously carried out.

(3) To define the epidemiology of Infertility, sexual health control programme is essential. Further, the role of infection in anoestrous condition needs to be investigated and sensitivity trials be carried out before resorting to antibiotic therapy of genitalia.

(4) The field of Male reproduction being new one, further detailed research work be carried out in this direction.

(5) Estimations of biochemical constituents be strengthened for better interpretation of disease conditions and laboratory facilities be provided for biochemical analysis, both at Universities and State Department levels. Further, the Normographs of foetuses in different Indian breeds be worked out for their utility in Medico-legal cases.

(6) The antisera for steroid and protein hormones be provided to all laboratories and Indian Council of Agricultural Research should sponsor research projects on production of antisera. Further, since the quality control is essential in such a type of work, the established laboratories should play a supervising pivotal role and assist in assay work of other centres too.

(7) In order to prepare specific immuno-agents, research on methodology for separation, characterisation and quantitation of cell types from adeno-hypophysis secreting various hormones be conducted and further pharmaceutical standardisation of Resorpine for inducing lactation be carried out.

(8) The I.C.A.R. be approached to provide financial assistance to different schemes for conducting extensive studies on production and reproduction traits.

(9) In order to have better facilities for the Veterinary College students, the veterinary polyclinics under the Agricultural Universities need not exist as separate and independent clinical departments but one staff member (Director of Polyclinic) may co-ordinate the activities by incorporating expertise from three disciplines viz. Medicine, Surgery and Gynaecology.

(10) The state Veterinary polyclinics should necessarily have a qualified Gynaecologist at district level veterinary polyclinic.

(11) ISSAR gratefully acknowledges the financial assistance of Rs. 5000 for holding 4th All India symposium on Animal Reproduction at Hissar and Rs. 3000 for publication of third volume of the Indian Journal of Animal Reproduction.

(12) There should be a separate panel for Animal Reproduction in the I.C.A.R. with an Assistant Director General (Animal Reproduction) for co. ordination of research in Animal Reproduction at the National level.

DR. D. P. VELHANKAR

Hon. Secretary, I.S.S.A.R.

Hon-Secretary's Report:

Report on the 5th. National Congress on Animal Reproduction held at G.B. Pant Univ. of Agril. & Tech. Pantnagar from 27th to 29th. Feb–1984

The Indian Society for the Study of Animal Reproduction (ISSAR) in association with the G. B. Pant Univ. of Agril. & Tech. and the Indian Council of Agril. Research organised the 5th. National Congress on Animal Reproduction at the Dept. of Obstetrics & Gynaecology, Faculty of Veterinary Science, G. B. Pant Univ. of Agril & Technology at Pantnagar, Dist:-Nainital (U.P.) from 27th Feb 84 to 29th Feb 84. The congress was attended by 134 scientist delegates in the field of Animal Reproduction from various Agri. Universities, National Institutes, State Departments of Animal Husbandry, Milk Federations, Insurance Companies, Pharmaceuticals and Remount Veterinary Corps.

The congress and the symposium was inaugurated on 27th. Feb 84 by the Chief Guest Shri. Kripa Narainji, Vice-Chancellor of G. B. Pant Univ. of Agri. & Technology, who laid stress for increasing the milk production through innovative research so that the goal "Milk to the million masses" be achieved. Dr. I. P. Singh, Pro-Vice-Chancellor & Dean Veterinary Faculty presided over the inaugural function.

Topics on Male and Female reproduction.

The topics on male and female reproduction were discussed in twelve technical sessions. In all 67 papers on female reproduction and 54 on male reproduction and reproductive efficiency were presented.

There were four guest lectures on the following topics:

(1) Endocrinology of male reproduction by Dr. M. L. Madan.

(2) Role of viruses in Reproductive disorders of bovines Dr. N. P. Yadav.

(3) Optimising reproductive efficiency in Buffaloes by Dr. K. Janakiraman and

(4) Sire selection for dairy cattle breeding by Dr. R. P. Chaudhury. The abstracts of the relavant papers have been published in Feb. 1984 issue of the I.J.A.R.

The technical sessions were followed by planary session wihch put forth 12 recommendations published elsewhere in this issue.

The symposium was a great success, ISSAR extends sincere thanks to Dr. S. N. Maurya, Organising Secretary and his colleagues in organising the Vth National Congress.

ISSAR Fellowship Awards:

The fellowship of the Indian Society for the Study of Animal Reproduction was bestowed upon the following dignitaries *for their meritorious contribution* in the field of Animal Reproduction.

Dr. D. P. Velhankar, Dr. S. N. Maurya, Dr. R. V. Patil, Dr. M. L. Madan.

The awards were presented by the Chief-Guest Dr. Kripa Narayanji, Vice-Chancellor, G. B. Pant Univ. of Agri. & Tech. at the inaugural function of the congress on 27-2-84.

Nils Lagerlof Memorial Award: 1982:

This award instituted by the ISSAR in memory of Prof. Emeritus Nils Lagerlof. 15 scientific papers related to the subject of Animal Reproduction and published during the year 1982 were received for the considerations of the award. In the opinion of the judging committee the following research publication was adjudged as the best. "Gonadotrophin Releasing Hormones in the treatment of anoestrous buffalo cows and heifers" by N. K. Khurana, R.P.S. Tyagi, R.C. Gupta and S.K. Verma. Published in the Indian Vet. J. 59, June 1982; 479-480.

The award was presented by Dr. Kripa Narayanji, Vice-Chancellor, G.B. Pant Univ. of Agri. & Tech. at the inaugural function of the 5th National Congress.

The Indian Journal of Animal Reproduction:

It is a matter of great satisfaction that ISSAR has been successful in bringing out the journal since 1981 and added the issues viz. Aug. 82, Feb. 83, Aug. 83 & Feb. 84 of the Indian Journal of Animal Reproduction. We express our thanks to the members of ISSAR Advertisers, I.C.A.R. & well wishers for their continued help & encouragement. The IJAR will be published henceforth in June and December months.

During the business session on 28-2-84, held in Dept. of Gynaecology G.B. Pant Univ. of Agril. Tech. New executive committee for the year 1984-87 was formed as under:

Dr. B. R. Deshpande	President
Dr. A. S. Kaikini	Vice-President
Dr. D. P. Velhankar	Hon. Secretary
Dr. M. L. Madan	Jt. Hon. Secretary
Dr. V. L. Deopurkar	Hon. Treasurer
Dr. S. B. Kodagali	Publication Editor
Dr. S. N. Maurya	Jt. Publication Editor
Dr. B. L. Bishnoi	Member
Dr. B. N. De	,,
Dr. C. P. N. Iyer	,,
Dr. K. G. Kharche	,,
Dr. L. D. Mohanti	,,
Dr. D. R. Pargaonkar	,,
Dr. R. V. Patil	,,
Dr. C. K. Rajkonwar	,,
Dr. R. Roy Choudhary	,,
Dr. I. M. Shah	,,
Dr. Tariq Ahmed	,,
Dr. S. K. Verma	,,

The ISSAR places on record the meritorious services rendered by Dr. C. R. Sane to the society. It was resolved to incorporate him as patron member. Similarly it was also decided to confer Hon. Life membership on Dr. C. R. Sane, Dr. S. N. Luktuke & Dr. G. B. Singh.

Sd:

(DR. D. P. Velhankar

Hon. Secretary

K.J. KAPADIA & CO.,
CHARTERED ACCOUNTS
167/71, DR. VIEGAS STREET,
GAIWADI, KALBADEVI,
BOMBAY: 400 002

Name: THE INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION

Address: _____ Accounting Year: 31st March 1984

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH, 1983

EXPENDITURE	Amount	INCOME	Amount	Amount
To Journal Printing (ICAR)	30,980.20	By I.C.A.R. Assistance:		
To Souvenir Printing		Symposium	5,000.00	
(Symposium-Hissar)	3,437.45	(For 4th Symposium at Hissar)		
To Delegates Lunch, Refreshments		Journal Publication (I.J.A.R.)	3,000.00	
(During Symposium at Hissar)	18,167.00	By Donations		8,000.00
To Decorations	504.25	By Delegate Fees		25,650.00
To Medals and Badges	80.00	By Membership Fees		12,300.00
To Postage and Telegrams	1,481.00	By Bank Interest:		
To Stationery	1,563.25	F.D.R. interest	3,109.33	
To Conveyance	520.15	Savings interest	622.03	3,731.03
To Bank charges	27.15	By Advertisements		18,335.00
To Audit Fees	251.00	By Sale of Journals		1,834.08
To Miscellaneous Expenses	519.05			
To Surplus of Income over Expenditure transferred to Balance Sheet.	16,335.51			
	Rs. 73,866.16			Rs. 73,866.16

For K. J. Kapadia & Co.

Sd/- Chartered Account 10.1.84

Sd/- President Sd/- Hon. Secretary Sd/- Treasurer

The Indian Society for the Study of Animal Reproduction (I.S.S.A.R.)

K. J. KAPADIA & CO.
CHARTERED ACCOUNTANT
167/71, DR. VIEGAS STREET,
GAIWADI, KALBADEVI,
BOMBAY 400 002

Name: THE INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION

Address: _____ Accounting Year: 31st March 1983

BALANCE SHEET AS ON 31ST MARCH 1983

LIABILITIES		Amount	ASSETS		Amount
TRUST FUND:			BANK BALANCE:		
Balance b/f.	17,174.34		In Current Accounts		
			Bombay	5,684.91	
Add: Surplus during the year.	16,335.51	33,509.85	Hissar	8,279.13	13,964.04
			CASH ON HAND:		
			Bombay	786.01	
			Hissar	5,070,47	5,856.48
			FIXED DEPOSIT:		
			With S.B.I.		5,689.33
			AMOUNT RECEIVABLE FROM I.C.A.R.:		
			Symposium	5,000.00	8,000.00
			Journal	3,000.00	
					Rs 33,509.85
		Rs 33,509.85			

For K. J. Kapadia & Co.,
Sd/- 10/1/84
Chartered Accountant

Sd/- President

Sd/- Hon. Secretary

Treasurer

List of Life Members of ISSAR

Sr.No.	Reg.No.	Name & Addresses
1	1	Dr. A.S. Kaikini B.Sc. (Vet. Hons, FRVCS (Sweden) Ph.D. Dean Faculty of Vety. Science Punjabrao Krishi Vidyapeeth AKOLA (Maharashtra) 444 104.
2	7	Dr. S.B. Kodagali M.V.Sc., FRVCS(Sweden), Ph.D. Professor of Gynaecology Veterinary College ANAND (Dist. Kheda) 388 001.
3	10	Dr. K.G. Kharche B.V.Sc., M.V.Sc., N.D.A.G., Ph.D. Asst. Prof. of Gynaecology Jawaharlal Nehru Krishi Vishwa Vidyalaya JABALPUR-482 001 (Madhya Pradesh).
4	16	Dr. B.C. Deka B.V.Sc. & A. H., M.V.Sc. Asst. Prof. of Gynaecology College of Vety. Science Khanpara, Gauhatti, Pin Code-781 022.
5	18	Dr. D. R. Pargaonkar M.V.Sc., FRVCS (Sweden), Ph.D. Professor of Gynaecology Faculty of Veterinary Science Marathwada Krishi Vidyapeeth PARBHANI (Maharashtra State).
6	36	Dr. S. H. Parekh B.V.Sc. & A.H. Artificial Insemination Officer Regional A.I. Laboratories Paddack Rajkot (Gujarat State).

2	2	3
7	59	Dr. B. R. Deshpande M.V.Sc., FRVCS (Sweden), Ph.D. Head of the Department of Animal Reproduction & Surgery Bombay Veterinary College Parel, Bombay-400 012.
8	80	Dr. D. P. Velhankar M.V.Sc., FRVCS (Sweden), Ph.D. Officer in charge, Veterinary Polyclinic Opp. Nirlon, Western Express High Way Goregaon (East), Bombay-400 065.
8a	82	Dr. C. R. Sane (Patron Hon. Life Member) G.B.V.C., F.R.V.C.S. (Sweden) 405, Naveen Asha Co-op. Hsg. Soci. 4th Floor, Dadasaheb Phalke Road Dadar, Bombay-400 014.
9	84	Dr. V. B. Hukeri B.V.Sc., M.Sc. (Vet), Ph.D. Professor of Gynaecology Bombay Veterinary College Parel, Bombay-400 012.
10	90	Dr. B. Shrinivasan Officer in charge Frozen Semen Bank, Hebbal, Bangalore-560 024 (Karnataka State).
11	95	Dr. V. L. Deopurkar M.V.Sc. Asstt. Prof. of Gynaecology Bombay Veterinary College Parel, Bombay-400 012.
12	97	Dr. S. R. Chinchkar B.V.Sc. & A.H., M.V.Sc. Asstt. Prof. of Gynaecology, Department of Gynaecology Bombay Veterinary College Parel, Bombay-400 012.

1	2	3
13	99	Dr. C. K. Rajkonwar B.V.Sc. & A.H., M.V.Sc, Ph.D. Associate Professor & Head Department of Gynaecology College of Veterinary Science Assam Agricultural University KHANPARA, Gauhati-781 022 (Assam State).
14	104	M. K. Tamuli B.V.Sc. & A.H., M.V.Sc., Dept of Gynaecology College of Vety. Science Assam Agril. University KHANPARA, Gauhati-781 022 (Assam State).
15	111	Dr. K. K. Bonia B.V.Sc. & A.H., M.V.Sc. Asst. Prof. A.P.M. Dept. College of Agriculture Assam Agri. University JORHAT-13 (Assam).
16	124	Dr. P. P. Jamkhedkar M.V.Sc., Ph.D. Veterinary Service Manager Pfizer Ltd., Express Towers 22nd floor, Nariman Point, Bombay-400 020.
17	140	Dr. S. N. Maurya M.V.Sc., Ph.D. (Illinois) Incharge Dept. of Gynaecology College of Vety. Sciences G.B. Pant University of Agriculture & Technology, Pant Nagar, Nainital-263 145 (Uttar Pradesh).
18	177	Dr. B. R. Benjamin B.V.Sc., M.Sc. (Man) Ph.D. Head, Animal Reproduction Division Indian Veterinary Research Institute IZATNAGAR-243 122 (U.P.).

1	2	3
19	195	Dr. K. K. Vyas G.B.V.C., F.R.V.C.S (Sweden) Emeritus Scientist, Shantiniketan, Purane Ginani Near Old Fort -334 001 BIKANER (Rajasthan State).
20	206	Dr. H. J. Derashri Assistant Professor Department of Gynaecoloy, Veterinary College, ANAND-380 001.
21	208	Dr. I. M. Shah Key Village Officer Directorate of A.H. Krishibhavan Paldi, Ahmedabad-380 006 (Gujarat State).
22	209	Dr. V. R. Jani A.I. Officer, Panchmahal Dist. Co-op. Milk Producers Union, GODHRA Dist. Panchmahal (Gujarat State)
23	211	Dr. Miss Lalita V. Deshpande B.V.Sc. & A.H., M.V.Sc. Reproductive Biology Research Unit Veterinary College, ANAND-388 001 (Gujarat State).
24	212	Dr. J. S. Patil Project Executive Animal Disease Diagnostic Lab. N.D.D.B. ANAND-388 001 (Gujarat State).
25	236	Dr. D. M. Patel Baroda Dairy Baroda

1	2	3
26	239	Dr. S. P. Banerjee Officer I/c. Polyclinic Veterinary Hospital Jaipur (Rajasthan State).
27	311	Dr. G. C. Jain M.V.Sc. Scientist D.C.N. & P. Division Animal Reproduction, N.D.R.I., KARNAL-132 001.
28	318	Dr. L. N. Purbey M.V.Sc. Associate Prof. of Gynaecology Division of A.R. I.V.R.I., IZATNAGAR Dist. Bareily (U.P.) 243 122.
29	319	Dr. B. L. Bishnoi Assoc. Professor, Veterinary College Bikaner (Rajasthan) 334 001.
30	327	Dr. L. D. Mohanty Dy. Director, Frozen Semen Bank Khapuria, CUTTACK-753 010 (Orissa State)
31	330	Dr. S. V. Rao Asstt. Director Frozen Semen Bank, Khapuria CUTTACK-753 010 (Orissa State)
32	365	Dr. P. A. Sarma Director, Central Frozen Semen Production & Training Institute Hessarghatta BANGALORE (North) 562 113.
33	367	Dr. K. P. Agarwal Asso. Prof. L.P.M. Vety. College Gujarat Agri. Univ. Sardar Krishinagar Dist. Banaskantha (Gujarat State)-385 506.

1	2	3
34	375	Dr. V. J. Pandya B.V.Sc. & A.H. Animal Husbandry Officer I.C.D.P. Surat (Gujarat State)
35	392	Dr. S. S. Chowsalkar D-66/1281, Azad Nagar Veera Desai Road Andheri, BOMBAY-58.
36	395	Dr. S. C. Sud Prof. of Animal Physiology College of Vety. Science G.B. Pant Univ. of Agril. & Tech. Pantnagar, Nainital-263 145.
37	397	Dr. Navneet Kumar Vasistha M.V.Sc. C/o. Shri B. K. Vasishtha P.F.S. Sakoh DHARMSALA (Kangra) H.P. Pin 176 215.
38	398	Dr. R. L. Dhoble M.V.Sc., Ph.D. Scientist S-I Division of Animal Reproduction I.V.R.I. IZATNAGAR (U.P.) 243 122.
39	399	Dr. S. K. Gupta M.V.Sc., Ph.D., Prof. of Gynaecology Division of Animal Reproduction IVRI, Izatnagar (U.P.) 243 122.
40	400	Dr. B. G. Hol. M.V.Sc. Farm Superintendent Cattle Breeding Farm NILELI, Post-Dukanwad, Tal. Kudal Dist. Sindhudurg. (M.S.).
41	402	Dr. M. L. Madan Ph.D. (U.S.A.) Prof. of Physiology N.D.R.I. Karnal-432 001.

Obituary

Biodata of DR. Bengt Lundgren

It is with great measure of shock that the veterinary profession, specifically the veterinarians involved in the discipline of animal reproduction in India will learn about the untimely death on 9-8-1983 of late Dr. Bengt Lundgren, Chief of the Artificial Insemination Association of Kalmar-Sweden. Dr. Lundgren was born on 21st March, 1916 and graduated from the Royal Veterinary College Stockholm in 1944. In 1945 he became Chief Veterinarian of the newly established Artificial Insemination Association at Kalmar and stayed there till the last days of his life. Under his dedicated stewardship Kalmar A.I. station developed to be one of the best in the country and supplied whole of the south-east part of Sweden with semen. He was a pioneer in the technical development of A.I. in Sweden and was one of the first to realize the advantages of the deep freezeing technique for bull semen.

Dr. Lundgren showed practical scientific approach to the problems of animal reproduction and detected a new type of gonadal hypoplasia and carried out research into its genetic origin. He also worked on infectious infertility in cattle and developed good hygienic and preventive measures against different types of reproductive disorders.

Dr. Lundgren worked hard to improve the quality of the Swedish red and white breed and was highly respected by the cattle breeders.

Internationally Dr. Lundgren came to India with Professor Lagerlof in 1953 and stayed behind for one complete year to train the veterinarians in Animal Gynaecology and Artificial Insemination technique.

In 1956-57 he taught animal reproduction at Cairo University. He worked as an FAO Animal Reproduction expert in Yugoslavia in 1972 and 1973 and in Egypt in 1975. As late as in 1981 he worked as FAO Expert in Sudan and Somalia. At the International Postgraduate Course on Animal Reproduction Dr. Lundgren was an extremely knowledgeable and interested teacher. Participants of each course since 1954 visited Kalmar and received unique love, affection and hospitality of the 'Lundgrens'.

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I Prof Dr SB Kodagali, Editor of THE INDIAN JOURNAL OF ANIMAL REPRODUCTION hereby declare that the particulars given above are true to the best of my knowledge and belief.

PROF DR SB KODAGALI
Editor
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