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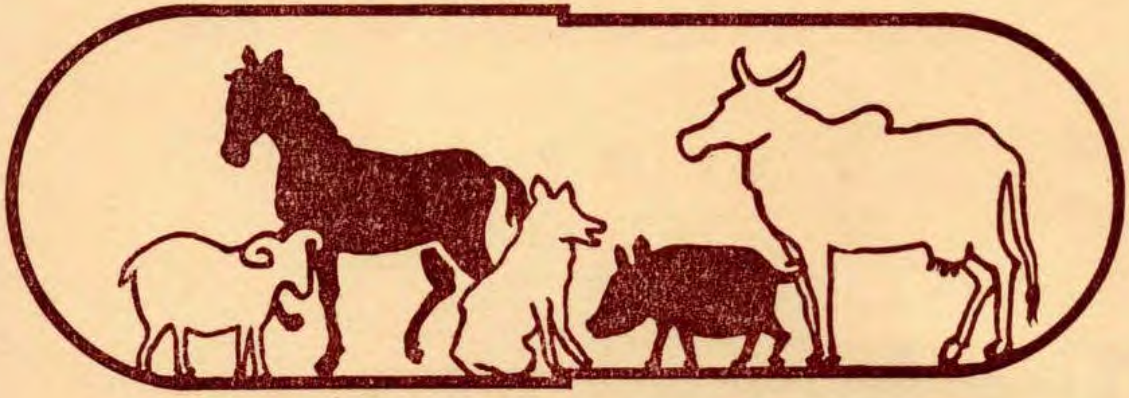

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The Indian Society For The Study Of Animal Reproduction

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THE INDIAN JOURNAL OF
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CONTENTS

Editorial

AN APPROACH TO FODDER DEVELOPMENT*

Fodder is the cheapest input which is converted into milk. Good quality fodder for the dairy animals is most essential and economical for feeding. Regular supply of green fodders throughout the year is very important for efficient animal production and reproduction. The problem of shortage of feeds and fodders is very well known. The cropping pattern that gives maximum dry matter and quality nutrients yield should be advocated. Many farmers produce feed and fodders by themselves. Milk production will become more economically viable depending upon how self-sufficient the farmers are regarding this input, as it practically covers around 70 to 80% of the total cost of milk production.

In the Key-note address delivered at this seminar by Dr. V. Kurien, Chairman NDDB, Anand and Vice-chancellor, G.A.U. stressed the need for a serious consideration to evolve a very practical approach to fodder development. The main constraints in fodder development are:

1. Non-availability of good quality seeds.
2. Lack of trained man-power.
3. A missing link between researchers and the farmers.

For doing this, he mentioned about the requirement of basic information on the present status of forage production area under important forage crops, average yields, condition of common grazing lands, natural grass lands, resources available with the farmers, existing feeding and fodder conservation practices, scope of introducing forage crops with grains & cash crops, extent of descript & nondescript fodder seed production and distribution in the state. He felt that such investigations are necessary to equip the planners, administrators, scientists and extension people to adopt a need based and practical approach to this problem involving "Farmer Power for the good of the Farmers themselves."

Some of the important recommendations drawn from the papers presented in the seminar delt with adequate storage facilities for fodder seed produced by farmers/seed growers; creating marketing infrastructure to procure seed at regular time and sell the same to farmers at subsidised cost; proper harvesting, baling and stacking of fodder/crops available during flush time; enriching the village grazing lands & Govt. waste lands by appropriate agronomic management practices; undertaking research work on grass-land development and silvipastoral management by the Regional Agricultural

Universities, ICAR Research Stations in collaboration with the concerned departments on the lines of work done by IGFRI, Jhansi and CAZRI, Jodhpur, and legislative cover for the village grazing.

The other items included in the report are: crop residues to be used only as feed; while evolving different varieties of agricultutral crops (dwarf varieties of cereals) fodder production should not be overlooked; for better utilization of roughages chaffing should be encouraged and incentives provided; treatment of crop residues to reduce the cost of production of milk such as of urea mollasses licks for enrichment; proper treatment of unconventional feeds before feeding them to livestock to augment the feed requirement; the fodder/grass/legume suitable for different districts/regions should be identified & recommended with appropriate cultivation practices; Fodder development programmes should receive high pricrity and sufficient allocation in the budget, and the area under the fodder crops be got indicated in the concerned proforma of village records.

Editorial Board

* SOURCE: Proc. of the Seminar on "Approach to Fodder Development in Gujarat" Organised by Dept. of Animal Husbandry, Gujarat State, at National Dairy Development Board, Anand on 22nd AUGUST 1984.

IJAR 5: 2: 1-7 1984

Heat Symptoms and Detection in Surti Buffalo Heifers

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ABSTRACT

Heat detection in buffaloes is more difficult than in cattle. This study indicates that a combination of the symptoms vulvar swelling and mucous discharge seems to be a relatively simple and reliable way of heat detection on farm level.

Other symptoms of oestrus were less common but can be a good supplement in heat detection.

Vaginoscopy and rectal palpation gave very important information and should be used in problem herds and individuals. The same was true for the fern pattern in dried vaginal mucus.

The progesterone levels in the peripheral plasma were below 1 nmol/l from two days before until two days after the day of oestrus. However, there are individual variations and the levels can remain high until 1-2 days before oestrus.

* * *

It is well know that the River buffalo in India has many advantages compared to crossbred cattle. The buffalo is more able to convert rough fodder cellulose into protein than is cattle, the fat content of the milk is higher, and the meat is accepted as human food amongst many people not accepting cattle beef.

The relatively low milk production of the buffaloes is depending on that very little breeding work and research was carried out until the last decades. Through milk recording and progeny testing there would be great possibilities to improve the production, knowing that at present a production of 3000 litres per lactation is not too uncommon and even 4000 litres per lactation have been recorded in several states of India (FAO, 1977).

The main obstacle in improving the dairy production is the low reproductive potential of the buffalo. This fact is depending on many factors; late maturity, long intercalving periods, seasonal breeding and poor heat expression. These factors have been listed by FAO as being of primary interest for intensive research.

Artificial insemination is a necessary tool for carrying out an effective breeding work, and if the heat detection is failing the artificial insemination will be unsuccessful.

As buffaloes in general show rather weak heat symptoms, the owners of the buffaloes as well as the AI technicians must have a good knowledge of those symptoms which are of importance. The problem has been that due to lack of knowledge many heat periods are missed and many buffaloes are diagnosed as having silent heat just because the symptoms may be hard to detect. A short list of some reliable symptoms, relatively easy to recognize if one is aware of what to look for, would be of great help to the farmers and the AI technicians.

Before 1970 there were few reports about research work on buffalo reproduction. Since then, however, the activities have increased considerably. Concerning heat symptoms in particular two important reports have been published recently Rao and Kodagali (1982) and Singh, Sharma Singh, and Sharma (1984). The subject has also been dealt with by several other authors Hafez (1954), Johari (1960), Gill, Gangwar and Kooner (1973a, 1973b), Janakiraman (1978), Rao (1979), Singh (1980), Rao and Kodagali (1983), Agarwal and Purbey (1983).

This report covers part of an on-going project to study the reproductive potential in Surti buffalo heifers. The project is carried out in cooperation between the AMUL Research and Development Association (ARDA) and the Swedish University of Agricultural Sciences

Materials and Methods

In all 29 Surti buffalo heifers were included in the study and were examined repeatedly throughout at least one sexual cycle. During the first investigation period, March 1980, 7 heifers were examined, during the second period, January — February 1981, 11 heifers and during the last period, November — December 1982, 11 heifers.

The clinical investigations were carried out at AMUL AI Centre, Kanjari, Gujarat State. Most of the heifers were purchased from small holders in the surrounding villages. Before the investigation period, the animals were kept at the station for 2—4 weeks and were given adequate feeding and management. During this time and the whole investigation period the heifers were kept loose in an open paddock, sheltered by trees The animals were given shower daily.

The animals were inspected 2—3 times per day, all of them in the morning and at noon. Those expected to approach heat were checked also in the evening.

The first group was palpated rectally every second day, the second and third groups were palpated every day. Vaginoscopy was used around heat and otherwise when found essential.

During heat a sample of the mucous discharge was examined for fern pattern under microscope (60x).

The rectal temperature was measured daily by means of a common fever thermometer. (For the first group an electronic thermometer was used, but it was found to be less exact and more time consuming than the common thermometer.)

Blood samples were taken every second day throughout one sexual cycle of each animal. The progesterone plasma levels were determined by radioimmunoassay (RIA) technique Bosu et al., (1976).

Results

Tne heat symptoms recorded at inspection and at special clinical investigation in the 29 Surti buffalo heifers are shown in table 1.

1. *Inspection*

Swelling of the vulva was found in all the buffalo heifers at oestrus. This was a reliable symptom provided the appearance of the vulva was compared to the conditions the previous days. There are great individual variations and therefore the size and shape of the vulva cannot be expressed in absolute figures but only relative to other days in the same animal. It is especially important to observe the wrinkles of the vulva.

As with swelling of the vulva, mucous discharge was found in all examined heifers at oestrus. The animals were checked very carefully for mucous discharge, also at nighttime when resting.

TABLE 1. Number of animals showing different heat symptoms

Heat symptoms	Period 1 n = 7	Period 2 n = 11	Period 3 n = 11	Total n = 29	% of total
1. Inspection:					
Swollen vulva	7	11	11	29	100.0
Mucous discharge at rectal palpation	7	11	11	29	100.0
Raised tail	1	5	6	12	41.4
Frequent urination	3	4	1	8	27.6
Bellowing	1	6	0	7	24.1
Mounting others	3	1	2	6	20.7
Standing for mounting	4	1	1	6	20.7
Restlessness	0	3	0	3	10.3
Investigating	2	1	0	3	10.3
Loss of appetite	0	0	1	1	3.4
Licking others	0	0	0	0	0.0
2. Special investigations:					
Fern pattern of mucous discharge	7	10	9	26	89.7
Vaginal hyperaemia	5	10	10	25	86.2
Contracted uterus	6	7	9	22	75.9
Open portio	2	7	8	17	58.6

TABLE 2. Fern pattern 1 day before oestrus until 1 day after oestrus in samples from the mucous discharge of n buffalo heifers.

Classification*	— 1 day n = 10		Oestrus n = 29		+ 1 day n = 18	
3	4	(40.0%)	15	(51.7%)	1	(5.5%)
2	4	(40.0%)	9	(31.0%)	6	(33.3%)
1	1	(10.0%)	2	(6.9%)	4	(22.2%)
0	1	(10.0%)	3	(10.3%)	7	(38.9%)

* Grade 3: Very characteristic fern pattern
Grade 2: Characteristic fern pattern
Grade 1: Less characteristic fern pattern
Grade 0: No fern pattern

The problem was, that the mucus was not always discharged spontaneously but sometimes only after rectal palpation or vagionoscopy. In the first two groups these animals were not recorded separately. In the last group, however, there were two out of eleven heifers which were recorded as not showing mucous discharge until rectal palpation was carried out.

Concerning the rest of the heat symptoms at inspection, table 1 shows that they were seen in a comparatively low number of animals. The third most frequently seen symptom was "raised tail". This symptom was checked after placing the hand on the rump of the heifer. In the 12 positive cases in the table there was a clear reaction with lifting of the tail and lowering of the back. In a number of additional cases there was a weak positive reaction.

Massage of clitoris will in cattle usually cause the same reaction as pressing the back in the lumbar region. However, in the present material of buffalo heifers it was found that they

reacted very negatively when the vulvar lips were touched.

Frequent urination and bellowing were seen in about one heifer out of four and was therefore less reliable as a heat symptom than the ones mentioned above.

About 20% of the heifers in heat would try to mount other heifers and an equal number of animals would allow mounting when they were in heat. No bulls were present among the experimental animals.

The remaining symptoms that can be observed at inspection were found in only 10% or less of the heifers.

2. *Special investigations*

Under the heading "special investigations" in table 1 some symptoms not possible to check by inspection have been recorded.

The fern pattern in dried mucous discharge as described by Agarwal and Purbey (1983) was classified into 4 different grades according to extent of crystallization and the results of the examinations are showed in table 2.

This method seems to be rather relevant for heat detection as 82.7% (24 out of 29) of the buffaloes had a very characteristic (grade 3) or characteristic (grade 2) fern pattern of the mucous discharge at the day of oestrus. Less characteristic fern attern (grade 1) was found in 6.9% of the samples and in 10.3% there was no fern pattern.

One day before oestrus there was mucous discharge in 14 buffalo heifers. Out of these the fern pattern was checked in samples from 10 buffaloes. In 80% the fern pattern was classified as grades 3 or 2.

One day after oestrus there was mucous discharge in 21 animals. Out of these the fern pattern was checked in samples of mucous discharge from 18 buffaloes. In 38.8% the fern pattern was classified as grades 3 or 2. It seems that the crystallization is more pronounced the day before oestrus than the day after.

Vaginoscopy provides important information about tne stage of the sexual cycle. During oestrus mucus will mostly be found in the anterior part of vagina as well as hyperaemia of the mucosa. In the present material hyperaemia was found in about 90% of the heifers. Portio was found to be open in about 60% of the oestrous heifers.

A well contracted uterus was found at rectal palpation in 22 out of 29 oestrous heifers. In a number of cases the oestrus contractions were intermittent, similar to those found in cattle at post-oestrus, although weaker. On the other hand, there were sometimes contractions also during midcycle.

The results of measuring the rectal temperature around oestrus were inconclusive.

3. *Progesterone determination*

The progesterone levels have been measured in blood samples taken every second day throughout one oestrus cycle in each animal. The amount of progesterone in the peripheral plasma 2 days before oestrus until 2 days after oestrus is shown in table 3. As can be seen from table 3 theconcentration of progesterone is close to zero during oestrus and during two days before and after oestrus.

However, one heifer had 10.6 nmol/l progesterone two days before oestrus but had 0 nmol/l on the day of oestrus. She behaved similarly the following cycle when she had 15.5 nmol/l three days before oestrus but 0 nmol/l two days later. The progesterone values can confirm the diagnosis of the stage of the sexual cycle.

TABLE 3. Progesterone levels in the peripheral plasma from 2 days before until 2 days after oestrus. (nmol/l)

	—2 days n = 13	—1 day n = 14	Oestrus n = 14	+1 day n = 15	+2 days n = 13
Mean	1.73	0.40	0.19	0.14	0.24
Range	0.0—10.6*	0.0—2.2	0.0—0.5	0.0—0.5	0.0—0.6

* Without this value the mean is 0.99 and the range is 0.0—2.5.

Discussion

From this study one can distinguish two symptoms of heat which are comparatively reliable for heat detection: swelling of vulva and mucous discharge.

One weakness of vulva swelling as a heat symptom is that it is sometimes hard to determine whether there really is a swelling or not. Thus, Singh *et al* (1984) reported from an investigation of 31 Murrah buffalo heifers that a pronounced vulva swelling was found in 18.47% and a slight/not evident swelling in 79.34%. However, the experience from the present study indicates that by close observation of the non-oestrus vulva of each animal it is possible to decide when there is a swelling. For a small holder in charge of one or two buffaloes only, it would not be difficult to differentiate between a swollen and a non-swollen vulva.

The mucous discharge as a heat symptom, although much easier to discover than the vulva swelling, has also got a weakness, as it is sometimes not occurring spontaneously. Although night inspections were carried out on resting buffaloes, using a torch and looking for a pool of mucous discharge on the ground underneath the vulva of the animals in heat, it was not always possible to find any discharge. In other investigations there are varying results reported. Sing *et al* (1984) found spontaneous mucous discharge in 100% of the examined Murrah buffaloes. On the other hand, Gill *et al* (1973b) reported a free flow of mucus only in 16.98% of the investigated Murrah buffaloes and Rao and Kodagali (1982) reported oestrous mucous discharge in Surti buffalo heifers in 56.29%.

A common disadvantage with both the discussed symptoms is that they will appear also during procestrus and post-oestrus. Especially this is true for the mucous discharge, as pointed out by Janakiraman (1978). Mucous discharge may occur 5 days before until 5 days after oestrus in a relatively large number of animals, Singh *et al* (1984). The difference in colour and consistency of the mucus during the various stages as described by many authors Singh *et al* (1984), Agarwal and Purbey (1983), Gill *et al* (1973a) would be a help in deciding the right day of insemination. This would probably be complicated for many farmers to learn but it is important that the AI veterinarians and the inseminators know how to classify the characteristics of the mucous discharge. In spite of the disadvantages these two heat symptoms in combination seem to be the most important ones for heat detection in buffaloes under field conditions.

Other symptoms of heat are important as complements to the previous ones. When observed, they will certainly help to discover the heat, but it is important to stress that no single sign by itself is enough for the diagnosis. Janakiraman (1978) found in a year-long study on 45

Surti buffalo heifers that frequent urination was never occurring during prooestrus but always during oestrus. Thus, he concluded that this symptom was the most specific and simple one in buffaloes. In other reports frequent urination was not found to occur to the same extent. In the present study frequent urination was found in only 27.6% of the animals. Singh *et al* (1984) found this symptom in 34.78% and Rao and Kodagali (1982) in 51.72%.

Concerning the special clinical investigations the fern pattern of the dried mucous occurred in about 90% of the heifers, but could not tell the exact day of oestrus. Out of the 90% positive about 7% were atypical while 10% were zero. Sukh Deo and Roy (1971) investigated the fern pattern in cattle and buffalo cows and related the typical, atypical and zero fern pattern to the pregnancy result after insemination. It was found that in buffaloes 75% of those with typical fern pattern became pregnant and only 37% out of those with non-typical pattern. In the zero-group only 5% became pregnant.

Similar results were found by Gill *et al* (1973a) and Agarwal and Purbey (1983) although in their reports there were no pregnancies in the groups with no fern pattern. Agarwal and Purbey (1983) reported 25% typical fern pattern, 45.83% atypical and 29.17% no pattern in 24 investigated buffaloes in heat.

Vaginal hyperaemia was found in about 86% of the animals in oestrus and open portio in 58.6%. The later figure seems low but in the remaining cases the portio was more or less oedematous. Rao (1979) reported that a fully open portio resulted in better conceptions.

The contracted uterus at heat occurred in roughly 3 animals out of 4. However, in the rest of the animals there was uterine tone during oestrus.

Vaginoscopy and rectal palpation as well as the fern pattern in the vaginal mucous are good methods and give reliable information for the heat diagnosis. They are very useful in problem herds.

Rao and Kodagali (1982) examined the vagina of 151 Surti buffaloes in heat. In 83·5% there were hyperaemia of the mucosa. Rectal palpation of the uterus revealed that 89% out of 500 buffaloes had a good or weak tone of oestrus.

A complicating factor in the diagnosis of oestrus in buffaloes is heat symptoms during midcycle. This phenomenon is well known to those experienced in buffalo reproduction, although not very much described in the literature. In this study one buffalo showed clear heat symptoms at day 8 of the cycle: spontaneous clear mucous discharge (no fern pattern), swelling of vulva, contracted uterus, open portio and hyperaemia of the vaginal mucosa. Another heifer had mucous discharge from day 9 to 11 of the cycle, fern pattern grades 1 and 2; a third buffalo showed mucous discharge, on day 9 of the cycle and a slightly contracted uterus. Only continnous examination in doubtful cases can help to eliminate a wrong diagnosis.

The "midcycle heat" is very confusing for the owner as well as for the inseminator. AI carried out at this stage of the cycle may cause infection of the uterus.

The low progesterone levels found at and around oestrus are in accordance with other reports. Jainudeen *et al* (1983) reported that the regression of corpus luteum after day 16 of the cycle in swamp buffaloes was accompanied by a sharp drop in the plasma progesterone level, reaching undetectable levels by the next oestrus. Similar conditions are

reported by Singh *et al* (1982), Arora and Pandey (1982), Batra *et al* (1979) and Perera *et al* (1978).

In cattle the pattern is the same Kindahl *et al* (1976).

There are, however, great individual variations. In this study one animal had high progesterone values one or two days before oestrus when there was a sharp drop. The same animal also showed a slow raise of progesterone in the peripheral plasma after oestrus.

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IJAR 5: 2: 8-14 1984

Non-damaging Technique for the Preservation of Living Cells at Low Temperature

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ABSTRACT

The authors present long term storage system for living cells, in way of experiment at the present time.

* * *

One of the fundamental laws for long term preservation is the slowing down of enzymes's function, so that cellular activity can be lowered, or even suspended, although tne components remain in full.

The preservation of sperm raised correctly tnis problem for the first time; it seems that this cell is adequate to sustain the experiments because she shows immediately and permanently, the life or the death and moreover, tne quality of life can be measured, by mean of the fertilizing power, daily evaluated in the centers of artificial breeding.

In theory, the conditions may be schematized in the equation of figure 1.

Figure 1.

Energetic substances in H_2O metabolites + energy (MOTION)
+ enzymes
Exogenous factors: Initial PH + varied PH
+ temperature
± microbes

Iuside the cell, one finds energetic substances converting in metabolites and energy, and outside, exogenous factors like the PH who can vary. That all happens in a small scale of temperature and can, eventually, be disturbed by microbes.

One can operate on the various terms of this equation and that is what was done in the artificial insemination.

First, by feeding energetic substances, one changes the data of tne equation from left to right, the sperm reaching again the initial energy level.

In secundo, from right to left, the advent of metabolites alters the PH and the buffers lengthen the duration of the preservation.

In third, by removing the water, an essential item in chemical reactions.

But experience proved although that lyophilisation impairs the survival of the spermatozoon.

One can also proceed, still from right to left, starting from the metabolites.

Indeed if the PH is not constant and varies, the spermatozoon stops his motion, even in case of an energetic excess, it suffers from its own autoantibiotic effect due to the lactic acid; the spermatozoon is not dead, but lives on without motion.

In fourth, one can accordingly make use of this autoantibiotism to stop the motion and extend thesurvival. By adding lactic acid, one stops the metabolism and by neutralizing this acid, it starts again. (Devuyst et Henriet 1962). We could in that manner maintain the sperm living, fully motionless, for 7 days and ascertain in due time the recovery of its motion. But after the fifth day, the survival was poor.

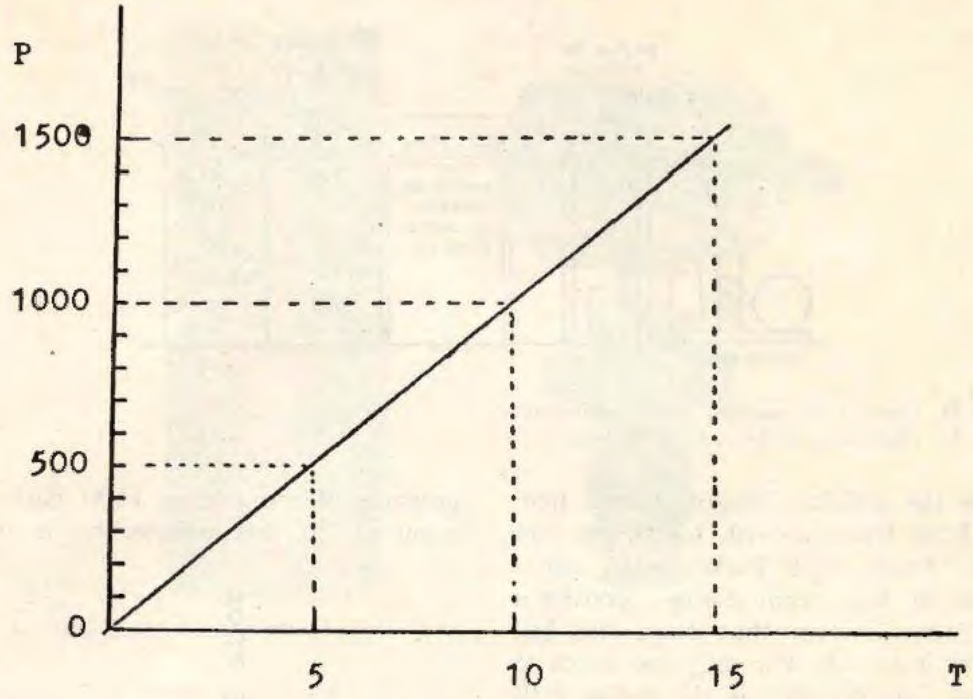


Fig. 2

In fifth, one can also work on the temperature. It is well known that the enzymes need for their function an optimal temperature. To change that temperature is equivalent to block the metabolism. The possibility of a raise is extremely limited, the proteins are highly thermo-sensitive and in all cases, an increase of the temperature increases the metabolism and shorts the life's time. Warm is good for food preservation, not for living material. Thus, we must inevitably go for lower temperatures. To succeed in this way, there was first to be found a protection against the cooling process: the egg yolk, who makes it possible to avoid the cold shock from $\pm$ 37°C to 4°C.

That is in short the way to preserve sperm, reckoning on the slowing down of the enzymatic activity. Antibiotics are needed, of course, to neutralize the exogenic factors of the equation. What is less well known is that energy can be saved by simple mechanical means: a conservation in a cold gelatine gel enabled us to regain spermatozoic motion after 23 days. More, we had to wait for 45 days to find all the spermatozoa dead in that gel. (Henriet 1964) This proves that the means of preservation are plentifull. But the slowing down is not enough to obtain an indefinite preservation: in the best diluents, at 0°C, the fertilizing power don't exceed 3 days.

Indeed, the preservation above 0°C is focused upon the energy equation. But, alas, quite a lot of otner chemical reactions are not inhibited in this way, so that we get damage very soon.

It may be noted that it is the fertilization power only that suffers, some spermatozoa still move after 3 weeks. But is it not just this fertilization power that

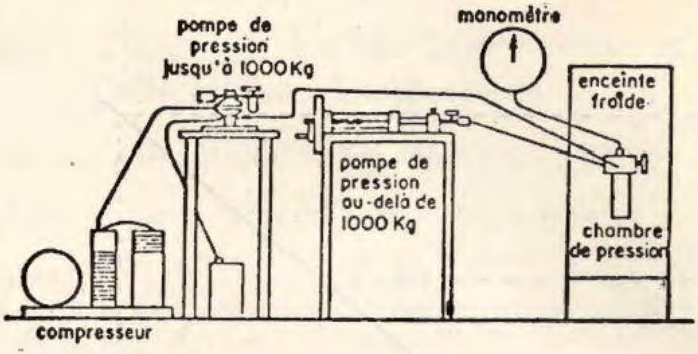


Figure 3: Plane of the material (first experiments)
Figure 4: Illustration of the starting material.

proves the cellular integrity? It is here that Jean Rostand with his froggs and Polge, Smith and Parkes with cattle success in long term storage, getting a congelation system that stops life but restores it at will. For this, one needs to use an "anti-freeze" as the living cells are essentially made up of water and cristallization must be avoided. Those "anti-freeze" do not always show advantages; the freezing impairs the fertilization power and many devices of antifreeze were tried out.

Aren't there other means to get under zero degree without "anti-freeze"? In physics, we know that water cristallizes at 0°C under atmospheric pressure. If we increase this pressure, the cristallization point lowers. We planned consequently a scheme to expose the cells to a sufficient pressure to thwart cristallization. But difficulcies arose immediately as the cohesion power of the atoms is heavy and increases extremely by a fall in temperature. A diagram of pressure and temperature of cristallization looks like this:

For each lowering of one degree Celsius we need a pressure of 100 Kg/cm² to prevent cristallization. Which means that to get a liquid stage of -15°C, a pressure of more than 1500 Kg/cm² is required. So this technic needs specizl

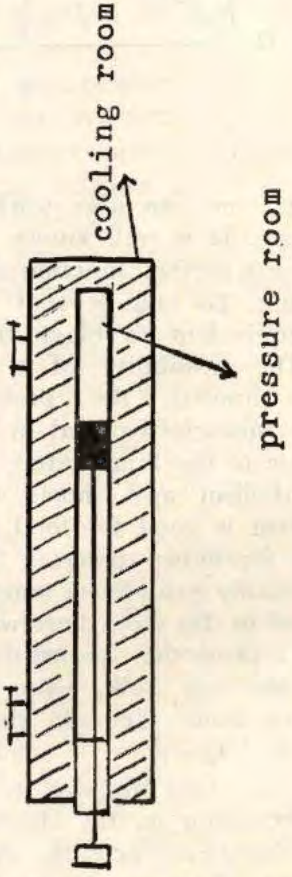


Figure 5: The light material.

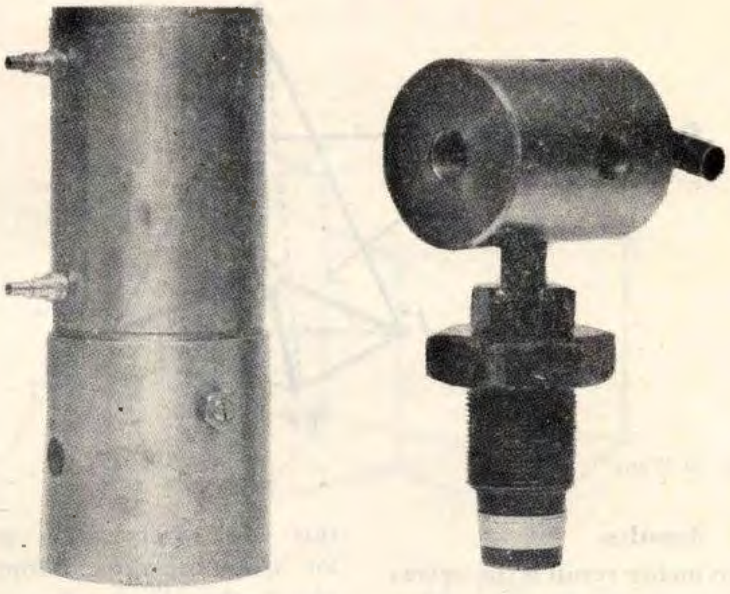


Figure 6: Illustration of the schema in figure 5.

apparatus.

From the left to the right: the compressor, the 2 pumps, the manometer, the cooling room and the pressure room.

The starting material is heavy, uneasy to manipulate, the caloric mass is enormous, involving a retardation in the pressure's steps and compromising a successfull storage. For this reason, the light system was built, reducing all the size.

Preservation attempts

a) 4 different kinds of cells were shut up together in the pressure chamber:—
de frosted sperm ready for insemination
— citrated blood
— mature oocytes
— and non mature oocytes both aspirated from follicles in follicular fluid.

b) containers: cells of each type were shut up in containers, not heavy containers, not with strong walls who don't convey the pressure to the fluid. As containers, we used so:
— Cassou's straws
— syringue with its piston
— rubber pear
— metallic ointment tubes.
All are suitable to endure high pressures.

c) freezing stages and pressure variations: The cells have to be put under pressure before crossing the zero degree Celsius and the apparatus nas to be cooled afterwards. But as the compression chamber has a high caloric mass we had to cool first up to 1°C-so as to avoid an uselessly prolonged enzymatic activity. The second stage is obtained after reaching necessary pressure. When the preservation has to be stopped, the opposite way has to be followed: pressure maintenance up to more than 0°C. Duration of our attempts: 5, 6 and 10 days.

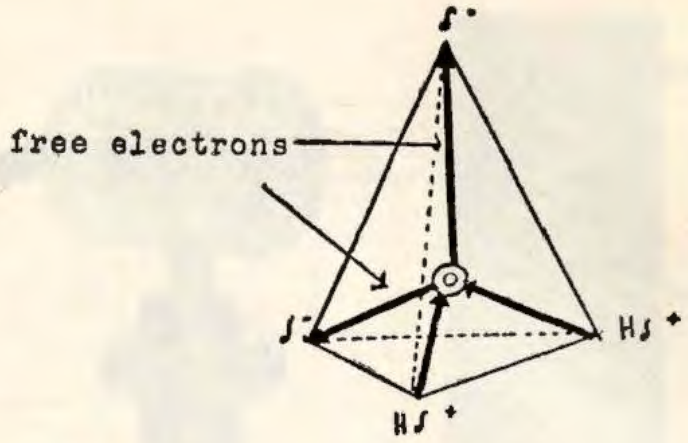


Figure 7. Structure of Water

Results

The most convincing result is the reversion of the cells to a normal type: neither the oocytes

nor the bloodcells (red + white)
nor the spermatozoa

looked impaired under the microscope. All those cells offer resistance to pressures up to 1600 Kg/cm² without bursting. Survival is although something else. The spermatozoa didn't move any more—they were dead. It is the big advantage of this cell type: we see immediately where we are. Now, we can reach 900 Kg without injurious effect.

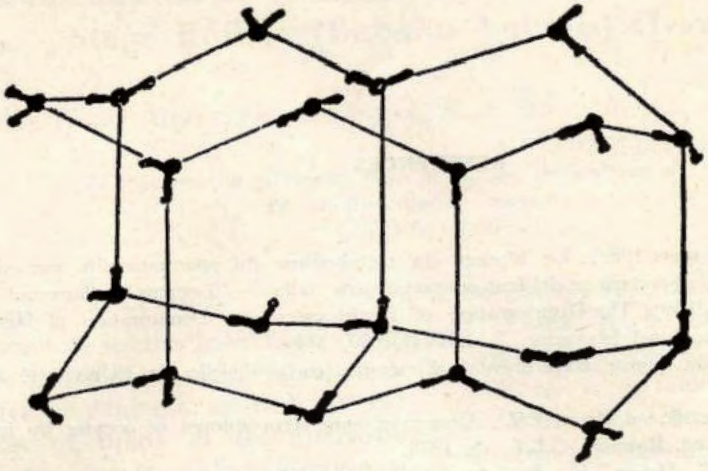
That is the case of the spermatozoa. But for the oocytes, it seems that this can be overpassed. They emerge out the system, unchanged.

The bloodcells were examined by a hematologist who, after the usual coloring, didn't find any impairment of the cells, red or white ones.

The future of this technique is now bound to a better understanding of the intracellular physics. (figures 7, 8 and 9). One finds three phases in water: solution, colloidal gel of protids and fats emulsion: this triple imbrication is unfavourable for the observator. Moreover, in each phase, the dipole of water plays in a strange manner: the molecules move quickly and their spatial order vary with the temperature from a tetraedric form to an hexagonal in "chair" or in "boat". The highest density is reached at four degrees and below zero, the angle of the dipole changes, the spacial order fills an enlarged volume. Tne motions stop, the 3 phases are quiet and the cells burst. When one adds glycerol, he acts on the angle of the dipole, and when we exert a pressure, we contrary the spatial order and the cristallization and in each case, the cells don't explode and the organelles are no more damaged. But, when the temperature decreases further down, the spatial ordering shrink without pressure.

It is then easy to conclude: if one can pass over the zone of large cristallization, until the zone of small, the pressure may be reduced or suspended without injurious effect on the cells. The zone of large cristallization is spread from zero to about twenty degrees under zero. At lower temperature, the zone of small

ice



oxygen
hydrogen

The hexagonal form in chair and boat.

Figure 8:

water

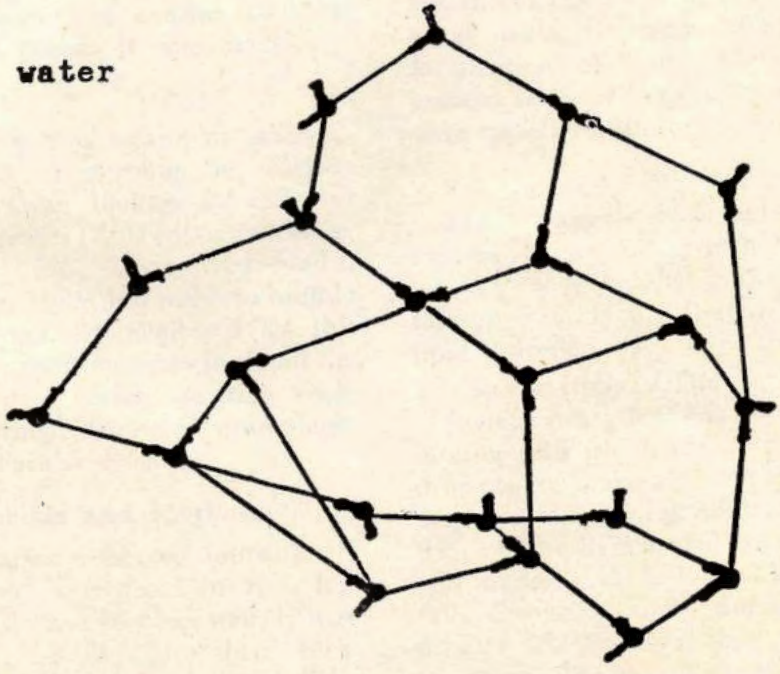


Figure 9:

cristalls is reached. It will say in a scale favourable, considering that usual material for cooling and pressure could be employed.

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Histochemical Studies On The Smooth, Preovulatory And Luteal Stage Buffalo (Bubalus bubalis) Ovary. I.

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ABSTRACT

Histochemical studies have been made of smooth, preovulatory and luteal stage buffalo ovary. Amount as well as the distribution of lipids in the interstital cells changes during different stages of the ovary. Interstitial cells are derived from the theca interna of the atretic follicles whereas luteinization of granulos cells, derived from the post ovulatory follicles, form corpus luteum. Both interstitial cells and corpus luteum undergo cyclization during the ovarian cycle as far as steriodogenesis is concerned.

* * *

Though there is a wealth of informations available concerning the various aspects of ovarian biology in different mammalian species (Zukerman and Weir, 1977; Jones, 1978; Motta and Hafez, 1980; Guraya, 1985) but hitherto buffalo ovary has remained neglected in this regard. This paper describes in detail the histochemistry of various ovarian compartments during different ovarian stages in buffalo (*Bubalus babulis*).

Materials and Methods

Buffalo ovaries, collected immediately after slaughter, were fixed in 10% formalin. On the basis of gross morphology ovaries were classified into three catagories: 1) Smooth (no ovulatory follicle, no corpus luteum) 2) Preovulatory and 3) Luteal stage. These stages were confirmed by staining 5-7 μm thick paraffin sections with haematoxylin-eosin. For histochemical locatization of proteins, mercuric bromophenol blue; proteins rich in-SH group and-SS-linkage, alkaline tetrazolium; carbohydrates, periodic acid-schiffs; mucopolysaccharides, aldehyde-fuchsin; lipids, sudan black B; phospholipids, acid hematin; cholestrol and its esters, schults method; triglycerides, Sudan III and IV and Nile blue sulphate were used (Pearse, 1972). For the localization of lipids 12-14 μm thick cryocut sections were used and reactions were confirmed after appropriate controls.

Results

The ovaries contained follicles at various stages of growth and atresia which were embedded in the stroma containing sparsely distributed sudanophilic lipid granules.

1. *Smooth Ovary:* The antral follicles of smooth ovary had less developed theca interna cells which contained small lipid droplets of variable size. These lipids consisted mainly of phospolipids and some triglycerides as revealed by acid hematin and Sudan III & IV. The granulosa cells contained small amounts of lipid droplets which were mainly phospholipids in nature. The granulosa cells of primary,

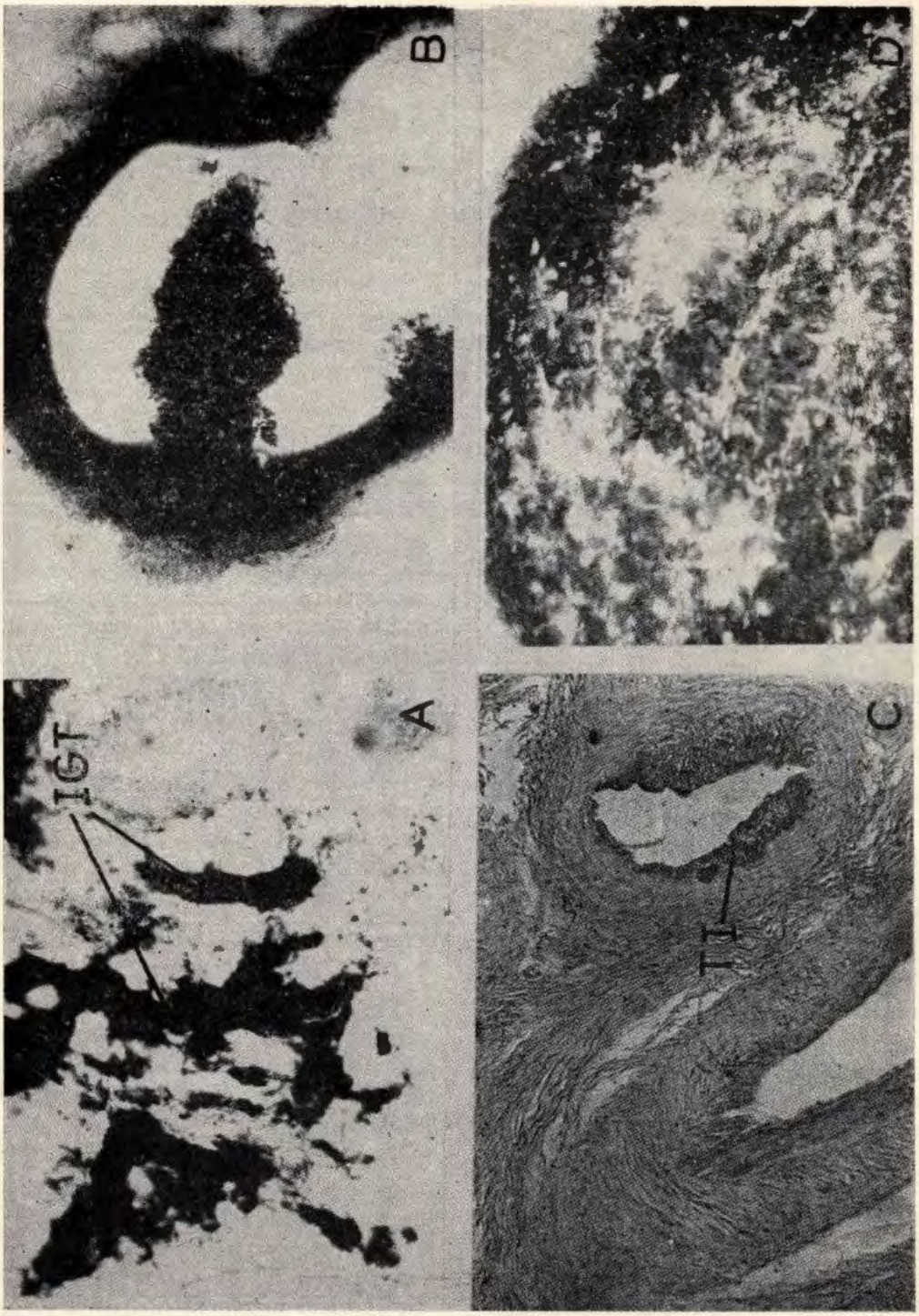

Fig. A. Smooth ovary showing very prominant interstitial cells (IGT) filled with sudanophilic lipids. SBB × 100.
Fig. B. Atretic follicle of preovulatory ovary containing abundant sudanophilic lipids. SBB × 100.
Fig. C. Theca interna cells (TI) of medium sized follicles of luteal ovary showing the presence of proteins. Bromophenol blue × 100.
Fig. D. Corpus luteum of luteal ovary containing sudanophilic lipids. Note some cells contain more where as others contain less lipids. Sudan Black B (SBB) × 100.

secondary, small antral and atretic follicles showed strong rection for protein with SH gp and -SS- linkages. Sulphated mucopolysaccharide revealed strong reaction in the granulosa cells of primary and secondary follicles but their stainability with aldehyde fuchsin decreased in the small antral and the antral follicles. The interstitial gland cells were very well developed (Fig. 1A). They formed thick and compact zones around the collapsed follicular cavity in the stroma of the ovary. The formation of these interstitial cells was closely related to the follicular atresia as they were formed from theca interna of the degenerating medium-sized and antral follicles in the surrounding stromal tissue. The fully differentiated interstitial gland cells showed relatively more lipid droplets and diffused lipids in comparison to the original theca cells as revealed by Sudan black B. The lipid droplets consisted mainly of phospholipids and some triglycerides, no cholestrol or its esters were localized with various histochemical techniques employed during the present study.

2. *Preovulatory ovary:* The theca as well as granulosa cells of preovulatory ovary contained small amounts of lipid droplets. The granulosa cells stained strongly for protein rich in-SH gp and -SS- linkages as well as for mucopolysacharides. The atretic follicles stained weakly for proteins and mucopolusaccharides with mercuric bromophenol blue and aldehyde-fuchsin respectively. The granulosa cells of atretic follicles stored sudanophilic lipids (Fig. 1B) which consisted of cholestrol, triglycerides and some phospholipids. The accumulation of sudanophilic lipids progressed at variable rates in different portions of the same atretic follicles. The granulosae of antral follicles stored relatively more sudanophilic lipids during atresia. The interstitial cells of preovulatory ovary contained relatively few lipid droplets as compared to the interstitial cells of the smooth ovary but contained small amounts of diffused sudanophilic lipids.

3. *Luteal ovary:* The luteal ovary showed histochemical features similar to the preovulatory ovary except for very well developed corpus luteum. The lutein cells of corpus luteum stained strongly for proteins but weakly for mucopolysaccharides. The process of luteinization involved gradual development of abundant diffusely distributed sudanophilic lipids in the granulosa cells. The amount of diffuse lipids varied greatly in individual cells (Fig. 1D) however, they contained few lipid droplets consisting of phospholipids and some triglycerides. The interstitial cells were less developed but they contained diffused sudanophilic lipids.

Discussion

Both corpus luteum and interstitial cells are the most important steroidogenic compartment of the ovary (Motta and Hafez, 1980). The present studies clearly reveal that both corpus luteum and interstitial cells undergo cyclization. When corpus luteum is well developed as in the luteal ovary, the interstitial cells are poorly developed where as, as in smooth ovary the interstitial cells are very prominent then there is no corpus luteum. Since the smooth ovary contains follicles at various stages of development and the folliculognesis is hormonally controlled process (Zukermen and Weir, 1977), it appears that interstitial cells provide the needed hormones and maintain their levels.

The granulosa cells appear to contribute to the major portion of the luteal cells as also reported by Guraya (1978).

The most striking and significant change during the transformation of granulosa cells into luteal cells is the gradual development of abundant diffusely distributed sudanophilic lipid in the later. Both ultrastructural (Motta and Hafez, 1980) as well as histochemical studies (Guraya, 1978, 1979) have shown that granulosa cells during the course of luteinization develops all the machinary required for steroidogenesis i.e. abundant endoplasmic reticulum, mitochondria with tubular cristae, sudanophilic lipids and the presence of 3 β-HSDH.

The formation of interstitial cells in the bovine ovary is closely related to the follicular atresia as they are formed from theca interna and surrounding stromal tissue of degenerating medium sized and antral follicles (Guraya, 1978). The interstitial cells form a thick and compact zone around the collapsed follicle. The fully differentiated interstitial gland cells show relatively more lipid droplets in comparison to the original theca interna cells. Lobel and Levy (1968) and Guraya (1978) have shown the presence of enzymes in the interstitial cells which are indicative of steroidogensis. Guraya (1978) has reported that the amount and the size of the lipid droplets is related directly to the intensity of their secretory activity as the interstitial cells which are producing steroid hormones very fast show relatively few lipid bodies of smaller size. That's why during present study the amount of lipids in the interstitial cells of preovulatory ovary decreases since hormone secretion is at its peak during the preovulatory stage (Motta and Hafez, 1980.)

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Circulatory Levels of Minerals Associated with Fertile and Nonfertile inseminations among Buffalo Heifers

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ABSTRACT

Circulatory levels of calcium, iron, zinc, copper, manganese and cobalt were estimated in buffaloe heifers following breeding. The average levels of calcium, iron, zinc, copper and manganese were 48.52±1.27, 1.04±0.05, 0.54±0.02, 0.76±0.02 and 0.01±0.00 ug/ml of plasma in fertile heifers which settled to insemination respectively. The corresponding mineral levels in heifers post nonfertile insemination were 46.31+1.06, 1.01±0.06, 0.65±0.04, 0.76±0.09 and 0.02±0.00 ug/ml, respectively. Cobalt was not detected in the plasma samples during the cycle. Variation in levels of the minerals u..der study among buffaloe post fertile and non-fertile insemination was not significant except for zinc, which was lower ($P < 0.05$) in pregnant buffalo-heifers. Comparatively lower levels of zinc were observed on days 1,3,17 and 21 post breeding in pregnant than in non-pregnant buffaloes.

* * *

Trace elements may function as co-factors, activators of enzymes, or stabilizers of secondray molar structure (Valee and Wacker, 1976). There has been special interest in effects of dietary trace elements on physiological functions in general and in reproduction in particular (Malecki, 1973; Hidiroglou, 1979). Circulatory levels of minerals vary, depending upon the stage of estrus (Dixit and Dubey, 1982) and following breeding due to the fast changes in the physiological status of animals. Circulatory levels of minerals in pregnant and non-pregnant animals may be taken as an index for their deficiency if any and their possible participation in the conception. Present work was therefore, undertaken to study the circulatory levels of certain minerals essential for reproductive performance and relate them to fertile or infertile insemination.

Materials and Methods

Ten healthy Murrah buffalo heifers, free from sexual and anatomical disorders were selected from the Institute's farm and managed under normal conditions of feeding and management. Concentrate ration was supplemented with necessary minerals and green fodder was fed *ad lib.* Blood samples from each heifer was collected before feeding at 8 AM on alternate day upto day 15 of estrus and daily thereafter upto day 22 post previous estrus. Blood samples were collected in heparinized glass vial by puncturing the jugular vein. Plasma was separated by centrifuging the blood at 3000 rpm for 30 min and preserved at —15°C till further analysis of minerals.

Thawed samples were analysed for calcium, copper, cobalt, iron, manganese and zinc using atomic absorption spectro-

TABLE 1: Circulatory levels of minerals (ug/ml) following breeding in buffalo heifers.

Day of estrus	Calcium		Iron		Copper		Zinc	
	P	NP	P	NP	P	NP	P	NP
0	50.48±9.65	46.45±4.30	1.10±0.21	1.07±0.08	0.67+0.05	0.73±0.11	0.60±0.12	0.80±0.24
1	53.40±6.15	41.30±4.08	1.00±0.26	1.33±0.39	0.72+0.14	0.67±0.11	0.70±0.19	0.99±0.34
3	39.00±3.52	41.63±4.04	1.10±0.15	1·28±0.24	0.78±0.06	0.75±0.06	0.52±0.07	0.92±0.36
5	53·83±8.21	48.03±6.99	0.70±0.07	1.36±0.31	0.92±0.05	0.77±0.09	0.47±0.06	0.57±0.07
7	48.00±5.20	45.84±1.75	1.03±0.19	0.95±0.08	0.80±0.09	0.65±0.10	0.55±0.04	0.56±0.04
9	51.20±6.06	46.48±6.04	1.15±0.19	0.82±0.07	0.83±0.05	0.80±0.10	0.59±0.06	0.53±0.02
11	53.38±6.41	45.84±8.84	0.78±0.13	0.78±0.17	0.63±0.11	0.78±0.51	0.48±0.12	0.53±0.09
13	43.88±2.51	47.76±8.52	1.10±0.20	0.72±0.19	0.94±0.11	0.54±0.11	0.46±0.05	0.56±0.10
15	53.08±9.83	58.36±5.33	1.33±0.35	0.92±0.10	0.66±0.08	0.65±0.08	0.57±0.07	0.56±0.08
16	46.55±6.55	51.22±4.57	—	0·85±0.16	—	0.71±0.16	—	0.54±0.13
17	49.03±6.11	49.17±4.44	0.88±0.29	0.77±0.09	0.69±0.13	0.46±0.01	0.55±0.05	0.78±0.27
18	41.04±7.37	46.88±0.69	0.95±0.20	0.86±0.11	0.73±0.08	0.61±0.10	0.47±0.06	0.47±0.03
19	54.34±4.35	50.00±4.60	1.45±0.65	1.33±0.29	0.72±0.02	0.76±0.15	0.46±0.02	0.63±0.09
20	51.70±10.29	43.33±3.26	0.80±0.14	1.37±0.42	0.75±0.11	0.61±0.21	0.44±0.09	0.42±0.03
21	39.05±5.50	47.18±5.94	0.93±0.03	0.65±0.07	0.64±0.08	0.52±0.05	0.70±0.06	0.91±0.32
22	47.65±4.91	41.53±2.53	1.27±0.15	1.03±0.23	0.70±0.05	0.78±0.18	0.53±0.03	0.60±0.03
Av.	48.52	46.31	1.04	1.01	0.76	0.76	0.54*a*	0.65*b*

P, Pregnant heifers, each value based on 4 observations.
NP, Non-Pregnant heifers, each value based on 6 observations.
—, not estimated
a,*b* differs significantly ($P<0.05$).

meter (Pye Unicam model SP 191) as reported earlier (Singhal and Mudgal, 1984). Estrus was detected by employing a vasectomized buffalo bull and the heifers were inseminated artificially twice at each estrus within an interval of 12 hours. Pregnancy was confirmed by rectal palpation at 60 days after insemination.

Statistical analysis of data was carried out according to Snedecor and Cochran (1968).

Results and Discussion

The circulatory levels of calcium, iron, zinc and copper at different days of estrus in heifer with successful (4) and unsuccessful pregnant (6) inseminations are presented in table 1.

Levels of calcium did not exhibit any pattern in pregnant and non-pregnant heifers. The average level of calcium (48.52 ug/ml) was higher in pregnant than non-pregnant heifers, however, their difference was non-significant. Lowest circulatory calcium level was observed on 3rd day of estrus which was followed by the highest level ($P<0.05$) on day 5 in pregnant heifers, whereas such variation was not detected in non-pregnant heifers. AICRPB Annual Report (1983) had similar observations in pregnant Surati buffaloes but their values were 2 to 3 times higher than values obtained in present investigations.

Average circulatory level of iron (1.04 ug/ml) was higher in pregnant than non-pregnant heifers, however copper levels (0.76 ug/ml) were similar in both type of neifers. The variation for iron and copper levels between both type of animals was non-significant. Highest level of iron and copper was observed on day

19 and 13 of estrus in both type of heifers, respectively. Since iron and copper are abundant in all foods (Singhal and Mudgal, 1984) of this area therefore, chance of deficiency in heifers seems rare, however, no clear relationship between copper in the blood of cattle and their fertility has been reported by Littlejohn and Lewis (1960) and Seekles and Claessens (1967). In some herds of cattle with low blood copper, female fertility has been impaired; yet in another conception rate has been high (Rowland *et al.*, 1977). Since the heifers were on similar ration with adequate copper content their circulatory copper levels remain similar and their low conception rate may not be attributed to the copper levels. Metabolism of copper appear to be altered during pregnancy. Copper in blood plasma of ewes falls during pregnancy and rises after parturition (Butler and Barlow, 1963). Contrary to present observations AICRPB Annual Report (1983) show extremely high and increasing levels of copper during the estrus and thereafter in pregnant buffaloes. Dixit and Dubey (1982) reported the lower levels of copper and iron than observed in the present investigation during various stages of estrus cycle in buffaloes and they also reported decreased level of copper at metestrus as compared to estrus whereas iron showed the opposite trend in same buffaloes.

The average circulatory zinc level (0.54 ug/ml) was lower ($P<0.05$) in pregnant heifers than non-pregnant heifers. Zinc levels were lower on each sampling day in pregnant heifers than non-pregnant heifers and the difference was pronounced during the first 3 days of estrus. Dufty *et al.* (1977) reported that zinc concentrations in plasma of non-pregnant heifers at various stages of estrus cycle were little changed. The lower levels of zinc during the first 3 days of estrus in pregnant heifers than in non-pregnant heifers, observed by us is difficult to be interpreted in terms of its causation. Though zinc deficiency has been attributed to result in lowered fertility, our observation with successful insemination; do not suggest the same. However, it needs to be qualified that in our case the sample size is small to have any influences in terms of fertility relation to zinc status. The levels obtained by us were higher than the values reported by Dixit and Dubey (1982), however, similar to present results they observed lower zinc level at metestrus than estrus in non-pregnant buffaloes.

The average circulatory level of manganese was 0.01 ± 00 and 0.02 ± 0.00 ug/ml in pregnant and non-pregnant heifers. The range of manganese was 0.00 to 0.03 ug/ml throughout the estrus irrespective of the group of heifers and their variation was non-significant. The Cobalt content was not detected in any of the blood sample, however cobalt is reported to be essential for the fertility (Hidiroglou, 1979).

It can be summarised from these studies that there was no marked difference in the circulatory levels of calcium, iron, copper and manganese in pregnant and non-pregnant heifers. The significantly lower ($P<0.05$) levels of zinc in pregnant heifers may be indicative of more spacific role of this element as a micro-nutrient, which needs further investigation.

Acknowledgement

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A Study on Placenta of Kankrej and Crossbred Cows

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ABSTRACT

With a view to study placental behaviour in Zebu and crossbred cows, 20 animals comprising 6 Kankrej (K), 9 Kankrej X Jersey-F_1 (KJ) and 5 Kankrej X Holstein Friesian-F_1 (KH) were studied. Results of the study indicated that the birth weight of calves in these groups differed significantly and highest weight was recorded for calves born to KH cows. There was no significant difference in placental size, number of cotyledons, size of cotyledons and time required for expulsion of placenta due to different genetic groups. The correlation coefficients between birth weight of calf and weight of placenta, birth weight of calf and time required for expulsion of placenta and placental weight and expulsion time were found to be non-significant between different genetic groups except in KH group where in the 'r' between birth weight of calf and time required for expulsion of placenta was positive and significant ($P<0.05$).

* * *

The third stage of parturition encompases the expulsion of foetal membranes and is a complex process involving both hormonal and mechanical factors (Clegg, 1959). This stage plays an important role in the succeeding breeding efficiency of the cow. Roy and Luktuke (1962) and Roberts (1971) have described the various stages of parturition. In the present study, an attempt has been made to study details of the shedding of placenta in crossbred cows (Kankrej × Jersey and Kankrej × Holstein Friesian) in comparison to that in Kankrej cows.

Materials and Methods

Twenty cows comprising 6 Kankrej (K), 9 Kankrej × Jersey-F_1 (KJ) and 5 Kankrej × Holstein Friesian-F_1 (KH) of the Livestock Research Station, Gujarat Agricultural University, Anand, were studied. Details of the animals selected have been furnished in Table 1.

The foetal membranes of these animals were collected immediately after calving, cleaned and weighed to nearest 100 g. The number of cotyledons and their diameter was recorded immediately. Time required for the expulsion of placenta was also noted. The weight of the calves, was taken immediately on drying and before feeding the colostrum.

The data were subjected to statistical analysis as per the method described by Snedecor and Cochran (1967).

Results and Discussion

Results with respect to placental behaviour and correlation between different factors are presented in Table 2 and 3, respectvely.

1. Birth weight of calves:

The average birth weights of calves in K, KJ and KH groups were observed to be 25.083 ± 1.589, 21.055 ± 1.415 and 28.600 ± 0.967 kg, respectively. The

TABLE 1: Details of the animals of different genetic group studied for third stage of parturition.

Sr. No.	Parameters		Genetic group		
			K	KJ	KH
1.	No. of animals		6	9	5
2.	Ave. age of animals on day of calving(days)		2492.67 (3)	1142.889 (9)	1421.80 (5)
3.	Ave. no. of lactations completed		3.167	1.750	1.500
4.	Ave. lactational yield (lit.)	Mean	1829.461	2852.100	4105.833
		SE	206.786	207.543	456.312
		CV%	47.950 (18)	19.253 (7)	27.218 (6)
5.	Ave. days in milk	Mean	265.333	316.571	392.833
		SE	19.559	14.191	15.910
		CV%	31.275 (18)	11.860 (7)	9.919 (6)
6.	Ave. dry days	Mean	149.389	87.429	78.833
		SE	17.883	11.462	14.760
		CV%	50.787 (18)	34.686 (7)	45.852 (6)
7.	Ave. calving interval (days)	Mean	415.278	404.000	471.667
		SE	19.124	17.236	24.806
		CV%	19.538 (18)	11.288 (7)	12.880 (6)
8.	Ave. last lactational yield (lit.)	Mean	2099.917	3124.875	4617.950
		SE	474.768	201.354	406.099
		CV%	55.380 (6)	12.887 (4)	21.536 (4)
9.	Ave. days in milk in the last lactation	Mean	265.500	337.500	415.000
		SE	49.916	18.549	9.174
		CV%	46.052 (6)	10.992 (4)	4.420 (4)
10.	Ave. dry days in the last lactation	Mean	174.500	94.750	91.250
		SE	27.450	18.309	19.648
		CV%	38.532 (6)	38.648 (4)	43.065 (4)
11.	Ave. last calving interval (days)	Mean	440.000	432.250	506.250
		SE	44.120	19.538	15.803
		CV%	24.560 (6)	9.040 (4)	6.243 (4)

Figures in the parenthesis indicate the number of observations per group.

K = Kankrej; KJ = Kankrej×Jersey (F_1);

KH = Kankrej×Holstein Firesian (F_1).

differences in birth weights due to different genetic groups were highly significant ($P<0.01$). The birth weight of calves in K and KJ genetic groups and K and KH group were similar to each other. Higher weights of calves born to KH animals may be due to comparatively larger body size of the animals. These results are in accordance with those reported by Patel *et al.* (1983) and Patel *et al.* (1984). Similar trend in birth weight of parental breed animals viz., Kankrej, Jersey and Holstein Friesian was reported by Tripathi *et al.* (1973);

TABLE 2: Placental behaviour and weight of calves in different genetic groups.

Sr. No.	Observations		Genetic groups		
			K	KJ	KH
1.	Ave. birth wt. of calves (kg).	Mean	25.083^{ab}	21.055^{a}	28.600^{b}
		SE	1.589	1.415	0.967
		CV%	15.513	20.166	7.560
2.	Ave. wt. of placenta (kg)	Mean	2.867^{a}	2.861^{a}	3.410^{a}
		SE	0.177	0.219	0.284
		CV%	15.110	22.933	18.617
3.	Ave. No. of cotyledons	Mean	84.000^{a}	76.222^{a}	94.200^{a}
		SE	11.424	6.808	10.007
		CV%	33.308	26.997	23.754
4.	Ave. diameter of cotyledons (cm)	Mean	5.152^{a}	4.536^{a}	4.624^{a}
		SE	0.426	0.218	0.250
		CV%	20.249	14.416	12.080
5.	Ave. time required for expulsion of placenta (hrs.)	Mean	6.167^{a}	4.528^{a}	4.300^{a}
		SE	1.123	0.271	0.700
		CV%	44.604	17.985	36.401

— K = Kankrej; KJ = Kankrej × Jersey (F_1); KH = Kankrej × Holstein Friesian (F_1)
— Figures bearing common superscript do not differ significantly from each other.

TABLE 3: Correlation coefficient among birth weight of calf, placenta weight and expulsion time in different genetic groups.

Sr. No.	Factors	Corelation coefficient (r)		
		K	KJ	KH
1.	Birth wt. of calf and wt. of placenta	0.745 NS	0.706 NS	0.541 NS
2.	Birth wt. of calf and time required for expulsion	—0.787 NS	0.144 NS	0.894*
3.	Placenta wt. and expulsion time	—0.568 NS	—0.068 NS	0.481 NS

NS = Nonsignificant * = Significant at $P < 0.05$.

Rajagopalan and Dave (1976) and Batra and Toughburry (1974).

2. Placental weight:

From Table 2, it could be seen that average weight of afterbirth in KH animals was higher (3.410±0.284 kg) than that in K animals (2.867±0.177 kg) and KJ animals (2.861±0.219 kg). However, these differences were found to be satistically non-significant. Similar results were also reported by Jose *et al.* (1984) and Patel *et al.* (1984). However, Bhosrekar and Sharma (1972) reported that weight of placenta is influenced significantly by genetic groups in cattle and buffaloes. Rao and Rao (1966) observed the weight of placenta ranging from 1.20 to 4.90 kg in Ongole cattle.

3. Number of cotyledons:

The average number of cotyledons in K, KJ and KH genetic groups was observed as 84.000±11.424, 76.222±6.808 and 94.200±10.007, respectively. Although KH group showed more number of cotyledons in afterbirth, it did not differ significantly from other two groups. These results corroborate with the findings of Dahiya *et al.* (1976) and Jose

et al. (1984). However, Bhosrekar and Sharma (1972) reported significant differences in number of cotyledons due to different genetic groups.

4. Size of the cotyledons:

The average diameter of cotyledons for K, KJ and KH groups was noticed at 5.152 ± 0.426; 4.536 ± 0.218 and 4.624 ± 0.250 cms, respectively (Table 2). These differences were non-significant. Hafez (1974) reported average diameter of cotyledons during pregnancy as 10 cms.

5. Time required for expulsion of placenta:

The mean duration for the expulsion of placenta was found to be 6.167 ± 1.123; 4.528 ± 0.271 and 4.300 ± 0.700 hrs., respectively for K, KJ and KH groups. Although, time required for expulsion of placenta in Kankrej animals is more than in KJ and KH animals, it did not differ significantly. Variation in time taken in Kankrej animals is quite high (44.64% as compared to other genetic groups. The observed values are in close agreement with those reported for various cow breeds by Clegg (1959), Rao and Rao (1966), Roberts (1971), Bhosrekar and Sharma (1972), Agasti *et al.* (1975) and Jose *et al.* (1984). Rao and Raso (1981) did not find any significant difference in length of time required for foetal membranes expulsion in Tharparkar and Brown Swiss x Sahiwal groups.

6. Association between different parameters:

The correlation coefficient between birth weight of calf and weight of placenta was found to be 0.745, 0.706 and 0.541 for K, KJ and KH genetic groups (Table 3). These values were not significant. Rao and Rao (1966) reported significant positive correlation between weight of calf and weight of placenta in Ongole animals.

In the present study, association between birth weight of calf and time required for expulsion of foetal membranes was negative but non-significant in Kankrej (-0.787) animals. But the values were positive both for KJ and KH animals. In KJ groups, it was not significant whereas in KH groups it was 0.894 and was significant ($P < 0.05$). Patel *et al.* (1984) noticed that birth weight of the calves did not affect the time required in expulsion of placenta in Kankrej × Jersey (F_1) and Kankrej × Holstein Friesian (F_1) genetic groups.

Placenta weight and expulsion time were negatively but nonsignificantly correlated in K and KJ groups whereas association was positive but non-significant in KH group. Similar non-significant relationship between these two factors was reported by Dahiya *et al.* (1974). Patel *et al.* (1984) observed tendency of heavier placenta to drop earlier in case of cows giving birth to Kankrej × Jersey (F_1) female calves.

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Serum Progesterone Levels In Buffaloes Retaining Fetal Membranes

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ABSTRACT

The serum progesterone (P) levels were estimated by radioimmunoassay on 285th, 295th and 300th day of gestation, on calving, and 12th hour, 1st, 2nd, 3rd, 5th and 10th days post-partum in 5 buffaloes retaining and 6 buffaloes not retaining fetal membranes (FM). The P levels in buffaloes not retaining FM continued to decline from 1.05 ± 0.11 ng/ml on 295th day of gestation to 0.18 ± 0.02 ng/ml on 10th day post-partum. In buffaloes retaining FM, the P levels on 285th day of gestaiton were 1.44 ± 0.44 ng/ml which declined to 0.16 ± 0.03 ng/ml on 10th day post-partum. The prepartum P levels, in general were found to be higher in buffaloes retaining FM than the buffaloes not retaining FM. The possibility of this high P concentration in buffaloes retaining FM resulting in an asynchrony of hormonal mechanisms that normally synchronize parturition and expulsion of FM is discussed.

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Retention of fetal membranes (FM) is a common post-partum complication which may reduce reproductive efficiency or even cause permanent sterility due to metritis, pyometra, salpingitis, etc. (Roberts, 1971). Retention of FM may be due to mechanical, infections, nutritional or hormonal causes. The present investigation reports the progesterone levels in buffaloes retaining FM.

Materials and Methods

Blood samples (20ml) were collected from the jugular vein of eleven buffaloes (5 retaining and 6 not retaining FM) on 285th, 295th, and 300th days of gestation, on calving and 12th hour, 1st, 2nd, 5th and 10th days postpartum. The animals were in their first to 6th lactation during the study. All the animals included in the study were kept under good feeding and managerial condition. The serum was separated from the clotted blood samples by centrifugation and stored at — 20°C until it was used for progesterone determination.

Serum progesterone was estimated by Radioimmunoassay (RIA) technique as described by Thorneycroft and Stone (1972) with minor modification. Aliqots of serum samples were extracted with 5 ml of diethyl ether on a vortex mixer for one minute. After extraction the aqueous phase was frozen and the ether extract was decanted in assay tubes. The extract was dried in water bath at 37°C. The dried contents were dissolved in 0.1 ml phosphate buffer saline (PBSG) (containing 0.1 M phosphate buffer, 0.9 per cent sodinm chloride, 0.1 per cent sodium azide and 0.1 per cent gelatin, (PH 7.0) followed by the

TABLE 1. Serum progesterone level (ng/ml) in buffaloes not retaining and retaining FM.

Groups	Sr. no.	Animal number	Gestation length (days)	Gestation days 285th	295th	300th	On calving	12th hour post-partum	Days postpartum 1	3	5	10
Buffaloes not retaining FM	1	716	305	1.20	1.20	1.12	1.20	0.16	0.36	0.42	0.18	0.26
	2	433	318	0.48	0.58	—	0.42	0.19	0.18	0.30	0.10	0.14
	3	981	308	0.36	1.40	1.30	0.24	0.24	0.24	0.20	0.20	0.14
	4	530	320	1.70	1.00	0.98	—	0.80	0.34	—	0.18	0.17
	5	634	310	0.86	1.10	0.82	0.50	0.34	0.49	0.24	0.20	0.22
	6	P—118	303	0.98	1.02	1.08	0.48	0.27	0.25	0.14	0.14	0.17
		Mean±S.E.		0.93 ± 0.20	1.05 ± 0.11	1.06 ± 0.08	0.57 ± 0.17	0.33 ± 0.10	0.31 ± 0.05	0.26 ± 0.05	0.17 ± 0.02	0.18 ± 0.02
Buffaloes retaining FM	1	949	305	1.80	1.60	—	—	0.42	0.32	0.20	—	0.20
	2	P—29	302	0.88	1.10	1.00	—	—	0.55	0.12	0.17	0.10
	3	P—249	309	1.10	1.50	0.80	—	0.25	0.33	0.35	0.36	0.12
	4	621	317	0.62	0.58	—	—	—	0.27	0.12	0.12	—
	5	P—323	295	2.60	0.70	—	0.70	—	0.65	0.58	0.14	0.20
		Mean±S.E.		1.44 ± 0.34	1.20 ± 0.23	0.90 ± 0.10	0.70 ± 0.00	0.34 ± 0.09	0.42 ± 0.08	0.26 ± 0.09	0.20 ± 0.05	0.16 ± 0.03

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

The within assay variance for different ranges of progesterone was calculated from 3 triplicate determinations. The coefficient of variation (CV) was found to be 8.52 per cent. The between assay variance was calculated from 3 duplicate low serum progesterone pool (serum from castrated male) and 3 duplicate high progesterone pool (serum from late gestation of cattle) and was found to be 17.19 percent. The standard curve for progesterone was linear in the range of 25 to 1000 pg.

Results

The serum progesterone levels in 5 buffaloes retaining FM and 6 buffaloes without FM retention are presented in Table 1. Although individual variations

existed, the mean serum progesterone levels gradually declined from 300th day of gestation till 5th day postpartum in buffaloes not retaining FM. However, the mean serum progesterone level on 5th and 10th day postpartum remained almost constant. The serum progesterone levels in buffaloes with retained FM also showed individual variations. The mean serum progesterone levels were found to decrease from 285th day of gestation till 10th day postpartum. The differences in serum progesterone levels between the groups of buffaloes retaining and not retaining FM were statistically non-significant.

Discussion

The serum progesterone levels in buffaloes not retaining FM continued to decline from 1.05 ± 0.11 ng/ml on 295th day of gestation to 0.18 ± 0.02 ng/ml serum on 10th day postpartum. Similar results were also reported by Dugwekar *et al.* (1975) and Rao *et el.* (1978). The progesterone levels in buffaloes reported in the present study were found to be in the same range reported by Ahmed *et al.* (1977) for pregnancy and luteal phase of the oestrus cycles in buffaloes. The individual variations in the progesterone levels within the group of animals retaining FM were observed making it difficult to draw a line between normal and abnormal on the basis of serum progesterone levels. Ahmed *et al.* (1977) also observed similar wide individual variations in the serum progesterone levels in buffaloes. The mean serum progesterone levels in buffaloes retaining FM were fouud to be 1.44 ± 0.34 ng/ml on 285th day of gestation. The serum progesterone concentration fell subsequently to reach a level of 0.16 ± 0.03 ng/ml on 10th day postpartum. There were no significant differences in serum progesterone levels between the animals retaining and not retaining FM. These results suggest that the estimation of serum progesterone levels alone may not be of clinical significance in the buffaloes with retained FM. However, estimation of serum progesterone levels along with serum estradiol and other related reproductive hormones in a larger number of animals with retained and non-retained FM might throw some light on the endocrine mechanisms involved in the process of parturition and the disbalance, if any, occurring in the hormonal levels resulting the retention of FM in buffaloes.

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Serum Progesterone Profile in Anoestrous Buffaloes and Cattle

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ABSTRACT

Blood serum progesterone estimation and per rectal examination was done to monitor ovarian activity in rural buffaloes and cattle having anoestrus characterised by smooth ovaries with no palpable follicles or corpus luteum. Low progesterone concentration (<1 ng/ml) and the smooth ovarian condition accompanied by anoestrus was noted in 4 of 5 cattle cows and in 4 of 5 buffalo cows, during the period of study (22 to 32 days). One buffalo cow showed a progesterone concentration of more than 1 ng/ml without a preceding palpable follicle or corpus luteum. One cattle cow showed an oestrous cycle without a preceding palpable follicle or corpus luteum.

* * * *

Anoestrus characterised by a per rectally palpable smooth ovarian condition without any follicle or corpus luteum is common in rural buffaloes and cattle of India (Luktuke and Sharma, 1978; Rao, 1982; Rao and Sreemannarayana, 1982). In the present investigation, a blood serum progesterone profile and per rectal examinations were done to assess the ovarian activity over a period of time in anoestrous buffaloes and cattle.

Materials and Methods

Buffaloes and cattle (F_1 generation of upgraded local cattle) presented with a history of anoestrus for month or more, and which had calved 2 to 6 months back were selected for this study. As per the history, earlier to the onset of this spontaneous anoestrus, these animals had shown at least one oestrous cycle. The study was conducted between November and February. These animals were per rectally examined at 4 to 7 day intervals. At the same time, 5 ml blood sample was collected through the jugular venipuncture. The separated serum was stored at −10°C pending analysis.

The progesterone concentration in the blood serum was estimated by a radioimmunoassay procedure using a specific antibody (Sheela Rani and Moudgal, 1977). Progesterone was extracted from 100 µl serum with 1 ml hexane. The extraction efficiency of the labelled progesterone was 74.5 ± 4.6% (n 10). Assaying a serum sample of low progesterone concentration, the respective inter- and intra-assay coefficients of variation were 17.1 and 10.4% (n 10). Assaying a serum sample of high progesterone concentration, the respective inter and intra-assay coefficients of variation were 18.2 and 23.1% (n 10). The water blank value for progesterone was 16.3±3.9 pg/ml. (n 5)

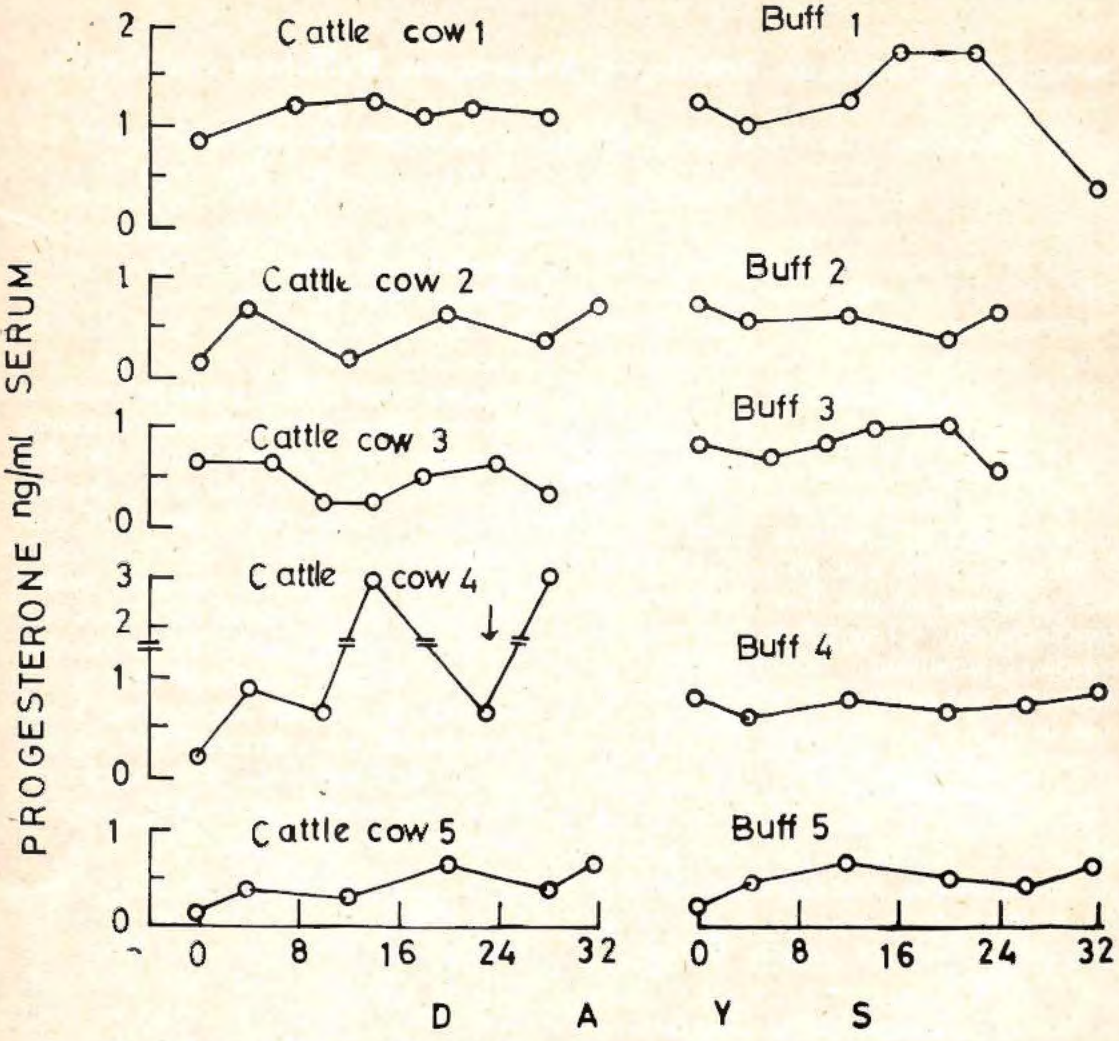

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Fig. 1: Blood serum progesterone concentration in buffaloes and cattle with an anoestrous syndrome. The arrow denotes the observed oestrus.

Results

On rectal palpation, all the buffaloes and cattle had a smooth ovarian condition without a follicle or corpus luteum during the period of study, i.e. 22 to 32 days. Excepting the buffalo cow 1 and the cattle cows 1 and 4, all others showed a serum progesterone concentration of less than 1 ng/ml (Fig. 1).

The cattle cow 4 showed oestrus with a peak progesterone of 23 ng/ml. Although the buffalo cow 1 had higher progesterone concentration (>1 ng/ml for 14 days, neither a follicle nor a corpus luteum was palpable.

Discussion

In the present study, in anoestrous buffaloes and cattle, there was a low serum progesterone concentration, accompained by a per rectally palpable smooth ovarian condition without a follicle or corpus luteum. The progesterone values of the present study are similar to those reported by Jainudeen, Bongso and Tan (1983) in buffaloes and Caudle, Thompson, Purswell, Sehrlin, Brooks and Smith (1982) in cattle with spontaneous anoestrus. In confirmity with the findings of Jainudeen *et al* (1983), in the present study, despite a high progesterone concentration in a buffalo cow and two cattle cows, there was a smooth ovarian condition. Perhaps, either there was an error in palpating a corpus luteum on the ovarian surface or there was a cryptic corpus luteum. This implies that in addition to the rectal examination, serum progesterone profile aids in arriving at a proper diagnosis.

The contribution of season as a factor for this anoestrous syndrome could not have been a possibility as the experiment was conducted during the winter, a period of normal oestrous activity in buffaloes (Rao and Pandey, 1982) and cattle (Jochle, 1972). Further studies are needed to investigate the factors associated with the spontaneous anoestrous syndrome in buffaloes and cattle.

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Attempts to Develop Solid-phase Radioimmunoassay of Buffalo Plasma Gonadotrophins

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ABSTRACT

Attempts were made to develop a solid phase radioimmunoassay (RIA) technique for estimation of gonadotrophin concentration in peripheral blood plasma of buffaloes. Despite a cross reaction between the buffalo pituitary Follicle stimulating hormone (FSH) and the ovine FSH, neither the buffalo plasma FSH nor the Luteinizing hormone (LH) concentrations could be measured. The concentrations of these hormones were below the sensitivity limits of the ovine FSH and LH assay systems. Hence there is a necessity to develop a sensitive homologous RIA for measuring the buffalo plasma gonadotrophins.

* * *

There are reports of having measured the LH and FSH in buffalo blood using ovine LH and ovine FSH systems (Sheth *et al.*, 1978 and Heranjal *ei al.* 1979 & Razdan *et a:.*, 1982), considering the cross reaction of antibodies against the ovine LH and ovine FSH with the buffalo LH and FSH. An attempt was made to measure buffalo plasma LH and FSH concentrations by liquid and solid phase RIA methodologies. Solid phase RIA is quick and permits the separation of bound and free lable to be performed by a rapid washing step. Catt *et al.*, (1968) and Goding *et al.*, (1969) have used antibody coated discs (for human LH) and tubes (for ovine LH) respecuively in the solid phase RIA. Ovine FSH antibody coated to sepharose was used in the present study.

Materials and Methods

Blood samples of 5ml. (50 i.u. heparin/ml. blood) were collected through jugular venipuncture from normally cycling buffaloes at 4 hour intervals starting from the time of onset of oestrus to 48 hour. The plasma was separated and stored at — 15°C until analysis.

Buffalo Pituitary FSH assay using ovine FSH systems: Buffalo pituitary was collected from the slaugher house and the adenohypophysis weighing 450 mg. was homogenized in 5 ml. sodium bicarbonate solution (pH 8). This homogenate was diluted to 1:2, 1:10, 1:100, 1:500 and 1:1000 and the FSH assay was run using all the above dilutions in the ovine system in solid phase RIA.

Assay of Buffalo Plasma FSH aand LH: For liquid phase RIA of FSH and LH methods of Moudgal and MadhwaRaj (1947) were followed.

Solid phase RIA for buffalo plasma FSH was done with ovine FSH antibody coated to sepharose and a 10% sucrose in 0.15 M phosphate buffer saline was used in the washing step that separated the bound from free label.

Results and Discussion

In the solid phase RIA of buffalo pituitary extract, a relative parallelism

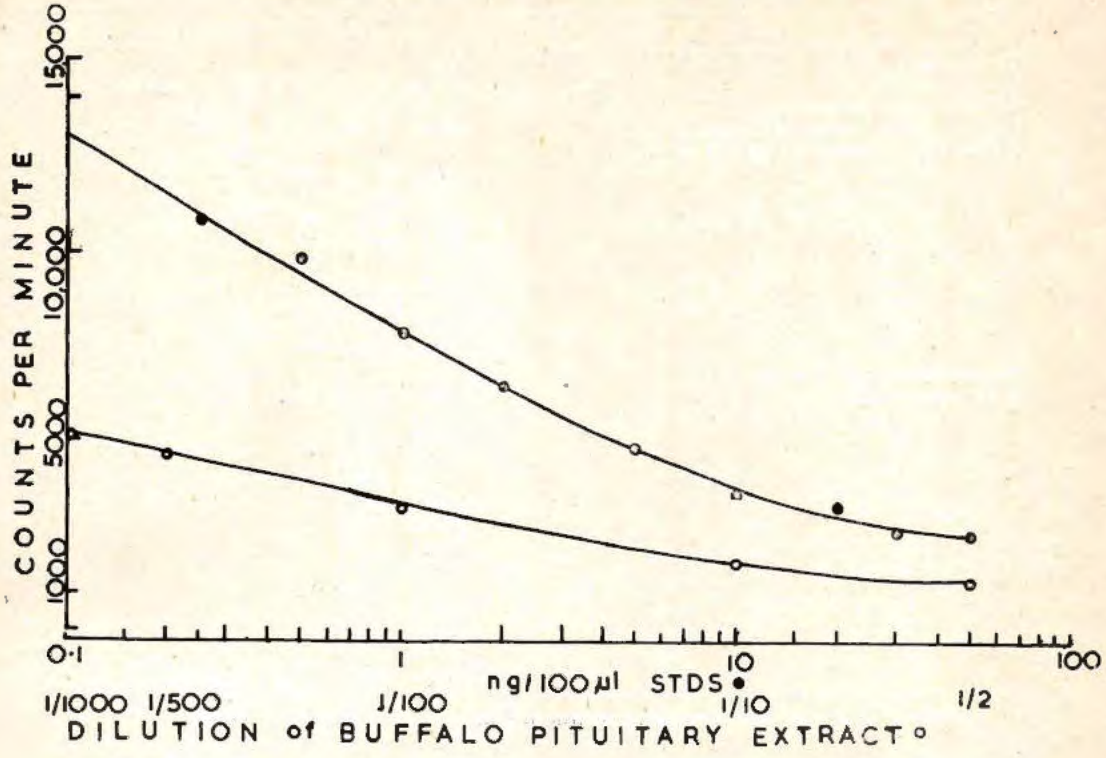

Fig. 1. Inhibition curves of buffalo pituitary extract and ovine FSH-NIAMDD-oFSH-13 in the FSH assay system by solid phase radioimmunoassay.

between the buffalo pituitary FSH and the ovine FSH was evident (Fig. 1). This indicated a cross reaction between the ovine and buffalo FSH. However, in the plasma samples neither the FSH nor the LH concentrations could be estimated since the cocentrations of these hormones appeared to be below the sensitivity limits of the assay system. (Tables 1,2 and 3).

The radioactive counts obtained for buffalo plasma FSH by the solid phase and liquid phase and for the LH by liquid phase RIA were above the specific binding and thus were outside the standard curve to be read (Tables 1,2 and 3). This suggests a possibility of low concentrations of LH or FSH in the buffalo plasma and a necessity to develop an RIA with greater sensitivity for the measurement of the buffalo gonadotrophins. Perhaps it could be advantageous to develop the homologous RIA for measuring the buffalo plasma gonadotrophins.

Acknowledgement

We thank Prof. N.R. Moudgal, Indian Institute of Science, Bangalore, for extending the RIA facilities at his department and the veterinarians at Buffalo Breeding Project, U.A.S., Dharwar for their co-operation and assistance in the collection of blood samples.

TABLE 1: Result of an FSH solid phase radioimmunoassay using the ovine FSH system

	Counts per minute
Total counts added	88643
Specific binding	13256, 13415, 12742 (14.81 percent)
Buffer non-specific binding	1812, 1264, 1454 (1.70 percent)
Buffalo plasma non-specific binding	12977, 12409, 14053 (14.83 percent)
Ovine FSH Standards ng/100 ul	
0.25	9880,11971, 10388
0.50	9850, 8535, 11115
1.00	7918, 7350, 8266
2.00	6613, 5646, 6583
5.00	4910, 4113, 4790
10.00	4412, 3645, 3635
20.00	3113, 3236, 3695
30.00	2669, 2460, 2858
50.00	2658, 2818, 2350
Plasma samples (100 ul)	
Intra-assay variation (n 6)	18804, 18674, 19925, 19202, 28284, 19292
1*	19899, 19002
2	16832, 17776
3	19210, 20809
4	16955, 18999
5	21642, 19959
6	20481, 17601
7	19302, 19561
8	20562, 22534
9	23236, 22545
10	19850, 17001
11	22579, 12792
12	24431, 19033

* Samples 1 to 12 were collected during oestrus at 4 hours interval from one animal.

TABLE 2: Result of an FSH liquid phase radioimmunoassay using the ovine FSH system

	Counts per minute
Total counts added	80336
Specific binding	55904, 54430 (68.68 percent)
Buffer non-specific binding	384, 3974 (9.78 percent)
Buffalo plasma non-specific binding	3049 (3.80 percent)
Ovine FSH standards (ng/100 ul)	
1	54443, 56074
2	56104, 53215
5	51636, 49191
10	45427, 46831
20	35875, 35756
30	28823, 29371
50	17609. 16214
100	7768, 7898
Plasma samples (100 ul)	
1*	53222
2	56002
3	55863
4	55624
5	52744
6	55972
7	55644
8	55949
9	55206
10	55185
11	55245
12	55703

* Sample 2 to 12 were collected during oestrus at 4 hours interval from one animal

TABLE 3: Result of a LH liquid phase radioimmunoassay using the ovine LH system

	Counts per minute
Total counts added	70526
Specific binding	13553, 14551 (19.92 percent)
Buffer nonspecific binding	5667 (8.04 percent)
Plasma nonspecific binding	3575, 4820 (5.95 percent)
Ovine LH standards (ng/100 ul)	
0.1	12469
0.2	9362
0.5	8366
1.0	7220
2.0	6005
5.0	5338
7.5	4378
10.0	4561
Plasma samples (100 ul)	
Intra-assay variation (n 5)	14162, 14063, 14192, 13764, 13296
1*	13515, 13366
2	13834, 14780
3	14093
4	14860
5	13635, 14421
6	13963
7	14392
8	13913, 13874
9	13346
10	14292, 15527
11	14541, 14421
12	14033, 14362

* Samples 1 to 12 were collected during oestrus at 4 hours interval from one animal.

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Clinical Investigation of Repeat Breeder Buffaloes for Ovulatory Disturbances

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ABSTRACT

A total of 477 oestrous cycles in 141 repeat breeder buffaloes were investigated for ovulatory disturbances. The incidence of delayed ovulation and anovulation was 16.56 and 15.09 per cent, respectively. Functional structures (CL and Follicle) on ovary were detected on clinical examination in 7.12 per cent cycles between 9 to 12 day of the oestrous cycles indicated midcycle follicular growth. The mid-cycle oestrus was observed in 10.69 per cent cycles of repeat breeder buffaloes. Clinical observations made in repeater buffaloes, for the study of oestrous signs encountered in 281 oestruses of 141 repeat breeder buffaloes were practically normal.

A total of 36 repeat breeder buffaloes were treated with ovulatory drugs. The drugs used were, chorionic gonadotropin hormone, progesterone and clomiphene citrate. The highest pregnancies (75.00%) resulted in group of animals treated with chorionic Gonadotrophin followed by 66.66 per cent in progesterone and 58.33 per cent in clomiphene citrate. The overall pregnacy rate was 66.66 per cent in treated and 30.33 per cent in control group of animals. The difference was found highly significant ($P < 0.01$.)

* * * *

The cyclic, functional or inflammatory changes in ovary can be detected relatively easily by clinical examination. Clinical changes in the bovine ovary are dependent upon and associated with periodic development of Graafian follicles, ovulation followed by development of corpus luteum and subseque regression of the corpus luteum accompanied by development of the new follicles. The gynaecological investigation of ovaries of repeat breeding buffaloes is of paramount importance. By extensive and careful examination of ovaries during oestrus and at regular intervals after oestrus, gives the idea of ovulatory pattern.

Materials and Methods

A total of 141 repeat breeder buffaloes and heifers were clinically investigated to study the ovulatory disturbances. Each repeat breeder animal was thoroughly examined on day of oestrus for the following aspects:

(i) Presence of external signs of oestrus like bellowing with-holding of milk frequent micturation, lumbosacral reflex, oedema of vulval lips and cervicovaginal mucus-discharge.

(ii) Rectal examination was carried out of location of "Graafian follicle" and "Regressing CL" on right or left ovary, degree of uterine tonicity

and degree of relaxation of external os.

After the careful examination the animal was inseminated. The second gynaecological examination was carried out after 24 hours on the same lines and if animal was found in oestrus and ovulation had not occurred the animal was re-inseminated. Third examination was carried out after 48 hours of first examination, if still the follicle was present, the case was diagnosed as of "delayed ovulation."

The same animal was called for examination between 9th to 12th day'post-oestrus for confirmation of ovulation and development of corpus luteum. If corpus luteum was not found on the ovary in which Graafian follicle was present the case was diagnosed as "Anovulatory oestrus". If only follicle was present on the same or opposite side ovary and obsence of corpus luteum along with uterine tonicity, cervical relaxation as well as other signs of oestrus the case was considered as of mid-cycle oestrus and insemination was carried out.

If both functional structures (CL and follicle) were present on the ovary along with weak signs of oestrus, the case was diagnosed as "Mid oestrus cycle follicular growth" and followed further for its cyclic nature.

When the cause of repeat breeding in the animal was diagnosed as either due to delayed ovulation or anovulation then during next or subsequent oestrous period the following treatment was carried out just after insemination in 36 animals.

In first group of 12 buffaloes 1500 I.U. of LH (Physex Leo, Decruz Corporation) was injected intravenously after insemination.

In second group of 12 animals with ovulatory disturbances were injected 25 mg of progesterone (Proluton-Depot, German Remedies) deep intramuscularly after insemination.

In third group of 12 animals, 10 ml of Clomiphene citrate (Fertivet-AR-EX Lab) solution was given intravenously after insemination.

All the above animals were re-examined between 9 to 12 days for development of corpus-luteum and followed for pregnancy results.

A total of 30 repeat breeder buffaloes (10 in each grup) were kept as control. In all control buffaloes inseminations were carried out during subsequent oestrouses and for pregnancy results.

Results and Discussion

A total of 477 oestrous cylces in 16 repeat breeder heifers and 125 buffaloes were investigated in the present study. The results have been presented in Table 1. The incidence of delayed ovulation in heifers and buffaloes was 18.86 and 16.27 per cent respectively. Anovulation was recorded in 20.75 and 14.38 per cent heifers and buffaloes respectively.

In bovines, the growth, maturity of GF and ovulation are regulated by pituitary gonadotropic hormones. Hormonal imbalance leads to defective ovulatory process. The manifestation may be delayed ovulation or failure of ovulation. Close co-ordination between the time of service and the time of ovaluation is essential. Thus these conditions can lead to repeat breeding conditions.

Roberts (1971) reported that there have been few studies regarding the ovulatory pattern and the disturbances in bovines. Hancock (1948) in his study on ovulation in cattle reported that 31 per cent cows had delayed ovulation.

TABLE-1: Ovulatory disturbances in repeat breeder buffaloes.

	No. of animals	Total number of oestrous cycle	Delayed ovulation	Anovulation	Presence of GF and DCL between 9 to 12 days of cycle	Presence of GF and absence of CL between 9 to 12 days of cycle i.e. Mid cycle oestrus
Heifers	16	53	18.86 (10)	20.75 (11)	5.66 (3)	9.43 (5)
Buffaloes	125	424	16.27 (69)	14.28 (61)	7.31 (31)	10.84 (46)
Total	141	477	16.56 (79)	15.09 (72)	7.12 (34)	10.69 (51)

Figures in parenthesis indicate number of oestrous cycle.

Key to abbreviations: GF = Graafian follicle, DCL = Developing corpus luteum, CL = Corpus luteum.

TABLE—2: Treatment of repeat breeder buffaloes with ovulatory disturbances and resulting pregnancies.

Drug used	No. of animals	Insemination number			Pregnancy per cent			Fertile oestrus interval (days)	Overall conception rate
		Cycle I	Cycle II	Cycle III	Cycle I	Cycle II	Cycle III		
Chorionic Gonadotrophin	12	12	4	3	66.66 (8)	8.33 (1)	—	2.50 ± 2.22	75.00 (9)
Chlomiphene citrate	12	12	7	6	41.66 (5)	8.33 (1)	8.33 (1)	8.85 ± 6.11	58.33 (7)
Progesterone	12	12	7	6	41.66 (5)	8.33 (1)	16.66 (2)	12.62 ± 6.63	66.66 (8)
Total (Treated group)	36	36	18	15	50.00 (18)	8.33 (3)	8.33 (3)	7.62 ± 2.96	66.66 (24)
Control group	30	30	30	26	—	13.33 (4)	16.66 (5)	31.88 ± 3.60	30.33 (9)

Figures in parenthesis indicate number of animals.

He also reported that the conception rate in ovulating cows was 65 per cent while it was 36 per cent in delayed ovulating cows.

Raizada (1981) reported 20 per cnt anovulatory oestrus in Murrah buffaloes. The observation on anovulation under the present study closely agreed with this finding.

Maree (1977) concluded that abnormal cycle length was associated with increased incidences of defective ovulation. She has examined 623 oestrous periods in 32 cows and observed 17.34 per cent delayed ovulation and 17.66 per cent anovulation. Under the present study the delayed ovulation was 16.65 percent

and anovulation 15.09 per cent. It is possible that the abnormal cycle lengths could be the cause of ovulatory disturbances due to the hormonal status. The hormonal factors need to be studied further.

During the period of study on ovulatory disturbances in repeat breeding buffaloes out of 477 cycles, in 34 (7.72%) cycles presence of Graafian follicle and developed courps luteum could be palpated during mid-cycle (9 to 12 days) and in 51 (10.69%) cycles presence of Graafian follicle only was observed in mid-cycle period. During the mid-cycle period under above two conditions oestrus signs were observed. The oestrus signs were pronounced in later situation and were mild in the former condition. The later condition could be termed as mid cycle oestrus and the former mid cycle follicular syndrome with non-functional corpus luteum. This is an important area for further probe. It is considered that the cycle length is shortened due to luteal deficiency (anovulation, ovulation but poor CL, corpus luteum that regresses by mid-cycle). In case of non-functional CL, the CL is palpable but could be non-functional. In view of the occurrence of mid-cycle oestrus, as no hormonal assays were possible under the present study no definite conclusions could be drawn. This area needs further investigations.

A total of 36 buffaloes were treated for delayed and anovulatory problems. In control group 30 huffaloes were kept. The results have been tabulated in Table 2. The results indicated an overall 24 (66.66%) buffaloes getting pregnant and out of 30 control buffaloes 9 (30.33%) conceived. The results were encouraging because of the previous diagnosis and subsequent treatment with the hormones and drugs to be ovulatory in nature.

The mean fertile oestrus interval in control animals was considerably longer (31.88 ± 3.60 days) when compared with the treated group (7.76 ± 2.26 days).

Hansel and Trimberger (1952) reported that small dose of progesterone during oestrus hastens the ovulatory process in dairy heifers. Thun *et al.* (1982) used peogesterone for treatment of repeat breeder dairy cows due to functional disorders with varying success. In the present study 12 repeat breeder buffaloes were treated with progesterone during oestrus just after insemination, out of which 8 (66.66%) conceived.

In the clomiphene citrate group, the pregnancy rate was 58.33 per cent. Clomiphene citrate is a proven ovulatory drug in case which do not ovulate due to a hypothalamic dysfunction. Evidence is also available that clomiphene has direct effect on the ovaries (Gemzell, 1975).

Tasi (1964) reported 77.50 per cent conception rate in repeat breeder cows with obscure causes when treated with follicle stimluating hormone and LH preparation.

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Total Carbohydrate Concentration in Uterine Secretions of Buffaloes During Certain Phases of Reproduction [1]

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ABSTRACT

The total carbohydrate concentration in uterine secretions of buffaloes during pro-oestrus, oestrus, dioestrus and early pregnancy is reported. There was cyclical variation ($P < 0.01$) in its concentration during oestrous cycle. Its concentration was significantly ($P < 0.01$) higher in dioestrus than pro-oestrus and oestrus. In early pregnancy, its concentration was significantly lower than in dioestrus. The carbohydrate concentration was 309.23 ± 12.78, 189.23 ± 18.91, 495.67 ± 17.06 and 350.13 ± 13.98 mg/100 ml during pro-oestrus, oestrus, dioestrus and early pregnancy respectively. The influence of gonadal hormones on the carbohydrate concentration in uterine secretions is discussed.

* * *

The importance of uterine secretion as a medium for sperm capacitation, embryo implantation and as a embryotroph to the developing conceptus has led many workers to explore its biochemical constituents. There are reports on the carbohydrate concentration in uterine secretions of cow (Heap and Lamming 1960; Ayalon 1978), ewe (Heap 1962) and rabbit (Heap and Lamming 1963). Where as there are no reports on the carbohydrate concentration in the uterine secretions of buffaloes. In the present study an attempt has been made to estimate the total carbohydrate concentration in the uterine secretions of buffaloes during certain phases of reproduction.

Materials and Methods

A total of 45 nonpregnant and 15 early pregnant uteri of healthy buffaloes were collected from the abattoir and transported to the laboratory in an ice packed thermocool box. They 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) and Abul Fadle *et al.* (1974) and in to early pregnancy (25 to 30 days) based on the biometry of the conceptus as per the technique of Arthur (1968).

The uterine secretions from nonpregnant uteri were collected by adopting the technique of olds and VanDemark (1957) with slight modification, where a roller pin was used instead of wringer clothe. In pregnant uterus a transverse incision just anterior to osinternus was made and the uterus was lifted from the tubal end for removal of the conceptus. A ligature was applied anterior to this incision and uterine secretions was collected similar to that of the nonpregnant uteri. The samples which contained cloudy flakes, and or blood tinge were discarded. Immediately after collection the samples were subjected for the quantitative determination of total carbohydrates by adopting the method of Dubois *et al.* (1956). The data was statistically analyzed

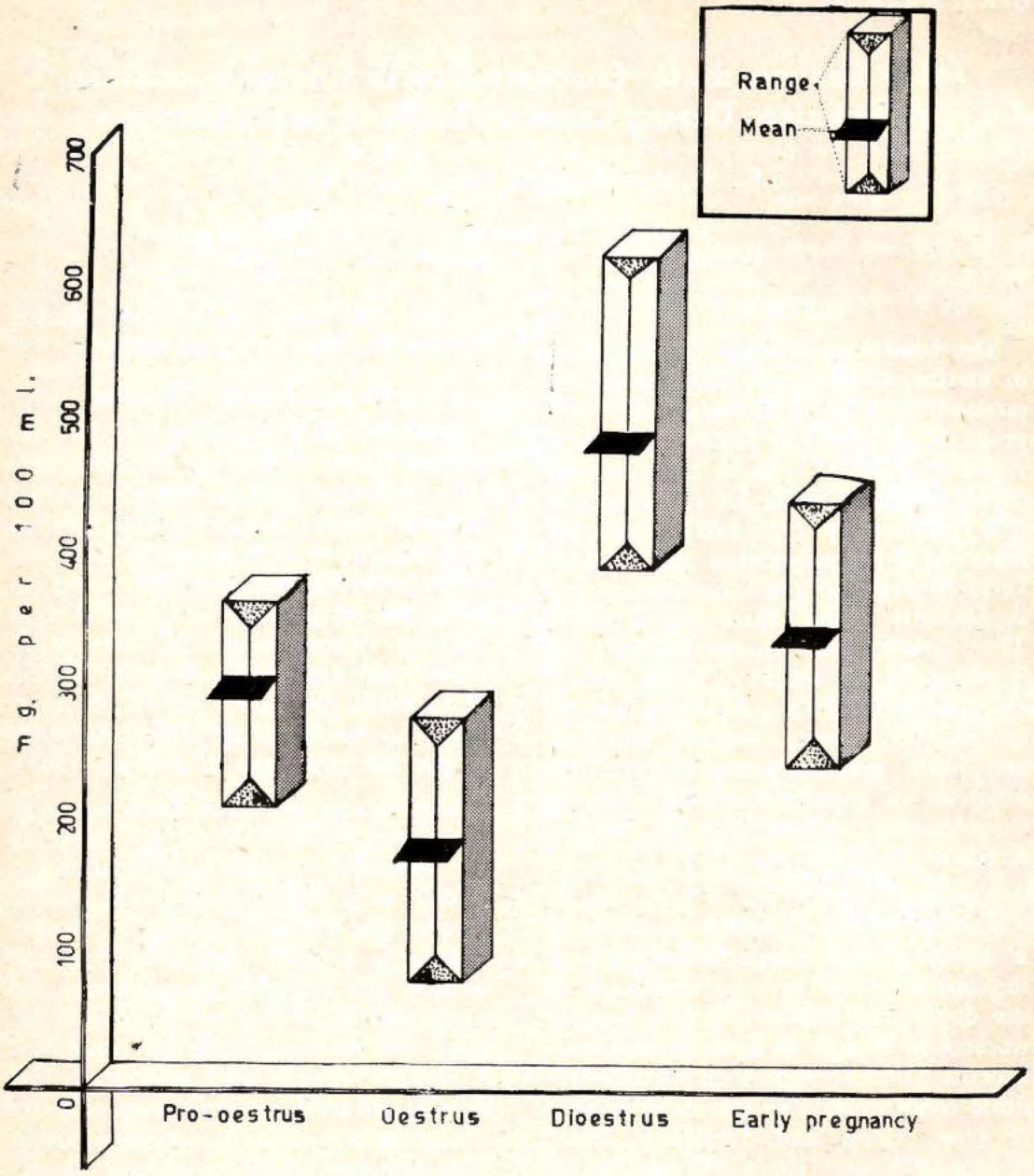

FIG. 1. TOTAL CARBOHYDRATE CONCENTRATION IN UTERINE SECRETION OF BUFFALOES DURING PRO-OESTRUS, OESTRUS, DIOESTRUS AND EARLY PREGNANCY

TABLE 1: Total carbohydrate concentration (mg/100 ml) in uterine secretions of buffaloes during certain phases of reproduction.

Reproductive Phase	n	Mean	S.E.	Range
Pro-oestrus	15	309.23[a]	12.78	212.50 to 378.00
Oestrus	15	189.23[b]	18.91	82.50 to 288.00
Dioestrus	15	495.67[c]	17.06	394.00 to 624.00
Early pregnancy	15	350.13[d]	13.98	246.00 to 526.00

Note: Figures with different superscripts varied significantly at 1 per cent level.

as per the method of Steel and Torrie (1960).

Results and Discussion

The mean concentration of total carbohydrate in uterine secretion of buffaloes along with range is reported in table-1. In oestrous cycle there was significant cyclical variation ($P < 0.01$) in uterine secretion carbohydrate concentration in buffaloes, with highest level in dioestrus and lowest level in oestrus. In early pregnancy its concentration was significantly ($P < 0.01$) lower than dioestrus (Fig.-1).

The biochemical constituents of uterine secretions showed cyclical variation in mammals. In rabbits, ewes and cows the uterine flushing carbohydrate concencentration was significantly higher in luteal phase than in follicular phase (Heap and Lamming 1960; Heap 1962). The results of the present study in buffaloes during oestrous cycle (fig-1) concurs with the reports of Heap and Lamming (1960) and Heap (1962) in rabbits, ewes and cows.

Gonadal hormones influence the biochemical constituents of uterine secretions. Progesterone therapy increased the carbohydrate concentration in uterine fluids of ewes and rabbits (Heap and Lamming 1960). Its concentration in uterine secretions of spayed rabbits reduced with oestrogen administration and increased with progesterone administration (Heap and Lamming 1963). The cyclical variation in buffalo uternine secretion carbohydrate concentration in the present study could be attributed to the influence of gonadal hormones and the significantly higher ($P < 0.01$) concentration of it in dioestrus than in oestrus and pro-oestrus is probably due to the influence of progesterone. Further investigations are needed to confirm this hypothesis.

The conceptus utilizes the energy substrate from uterine secretions for its development. The developing zygote which enters the uterus by 4 or 5 days after conception in bovines (Roberts 1971), utilizes not only its stored nutrients but also energy substrate from uterine milk for 33-36 days of gestation when haemotrophic type of nourishment is established in cows (Greenstein and Foley 1958). In uterine secretions of cows glycogen is a source of energy for the free floating blastocyst during 8 to 14 days of gestation (Larson *et al.* 1970). The lower concentration of total carbohydrate in uterine secretions during early pregnancy than dioestrus phase in the present study could be attributed to the utilization of carbohydrate by the developing conceptus.

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Presence of Decapacitation Factor(s) in the Seminal Plasma of Buffalo (Bubalus bubalis)

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ABSTRACT

Effects of different concentatrions of buffalo seminal plasma i.e. 5%, 10%, 15% and 20% on percentage motility, pattern of motility capacitation and acrosome reaction were studied. Ejaculated washed spermatozoa could be successfully capacitated in BWW mediun supplemented with BSA and SFMF (s) pH 7.4 and 35×10^7 s/ml concentration in the presence of 5 mM Ca^{2+}. The spermatozoa incnbated at various concentrations of seminal plasma viz. 15%, 10%, 15% and 20% showed a progressive decrease in percentage motility with increasing time of incubation. Increasing concentrations of seminal plasma i.e.5%, 10%, 15% and 20% increased the circular motility and decreased the forward mo.ility of spermatozoa. Higher concentrations of seminal plasma i.e. 15% and 20% were more detrimental. Various concentrations of seminal plasma significantly lowered the percentages of various stages of acrosome reaction i.e. acrosome swelling, acrosome vesiculation and acrosome shedding. Twenty percent seminal plasma inhibited the acrosome vesiculation, whereas 10, 15 and 20% seminal plasma completely inhibited the acrosome shedding.

* * *

Seminal plasma constitutes a beneficial environment for spermatozoa and it functions to transport and sustain their motility.[11,12] However, a number of studies have shown that seminal plasma can exert a direct toxic effect on epididymal spermatozoa[1,6,7,8,13]. Beas *et al.*[1] reported that bull seminal plasma contains separate factors such as motility stimulating factors(s) in the low molecular weight fraction and those having damaging effect on spermatozoa in the high molecular weight non-dializable fraction. One of the most important effects of seminal plasma is to induce decapacitation state in ejaculated spermatozoa. Several studies have shown the presence of decapacitation factor (s) in seminal plasma of various mammalian species[1,3,4,5,8,9] But there is no report of decapacitation in buffalo spermatozoa, therefore, taking this into consideration, we have studied the effects of different concentrations of seminal plasma on motility, capacitation and acrosome reaction of ejaculated buffalo spermatozoa. We have successfully induced capacitation and acrosome reaction in ejaculated buffalo spermatozoa *in vitro*.[14]

Materials and Methods

The buffalo semen was collected from fertile males with the help of artificial vagina. The ejaculated semen was maintained at 30°C and transported to the laboratory within 15-20 minutes.

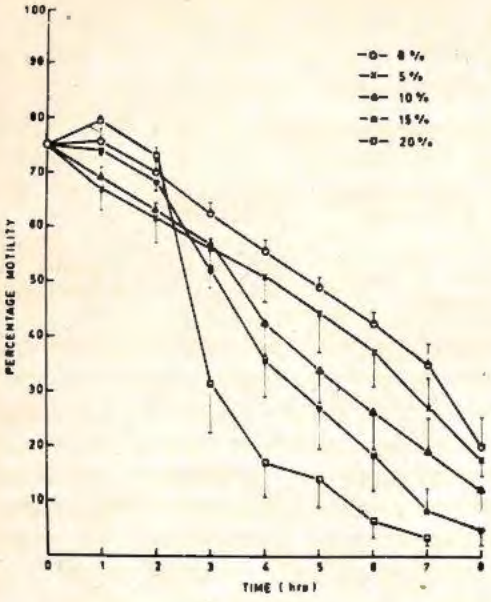

FIG.1. EFFECT OF DIFFERENT CONCENTRATIONS OF SEMINAL PLASMA ON PERCENTAGE MOTILITY (MEAN± S.E.) OF EJACULATED WASHED BUFFALO SPERMATOZOA

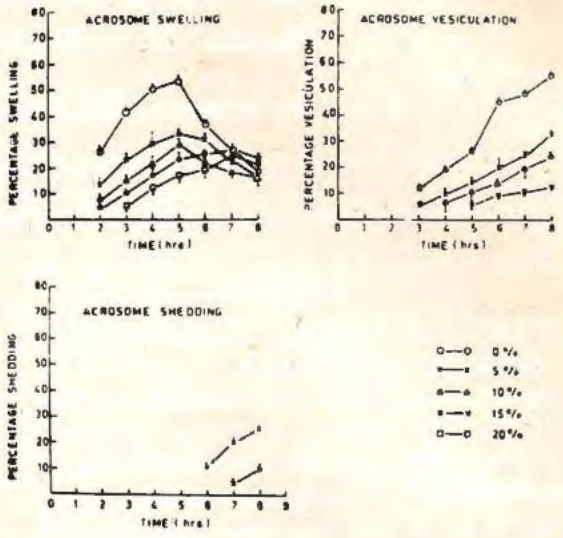

FIG 2 EFFECT OF DIFFERENT CONCENTRATIONS OF SEMINAL PLASMA ON DIFFERENT STAGES OF ACROSOME REACTION (MEAN± S.E.) OF EJACULATED WASHED BUFFALO SPERMATOZOA

Washing of spermatozoa for capacitation

Aliquots of ejaculated semen were centrifuged in a clinical centrifuge with swing out rotor at 500 g for 2-3 minutes at room temperature. The supernatant (seminal plasma) was aspirated by Pasteur pipette. The loose pellet of spermatozoa was washed twice, once with Biggers, Whitter, Whittangham (BWW) medium and then with hypertonic slaine (12 mg NaCl/ml). The washed pellet was gently suspended in BWW medium supplemented with bovine serum albumin (BSA) and sperm forward motility factor(s) (SFMF(s) as described earlier.[14]

2. *Effects of seminal plasma on motility, capacitation and acrosome reaction*

Seminal plasma was collected by centrifuging the buffalo semen through 1M sucrose at 10,000 g for 20-25 minutes at 40°C. Different concentrations of seminal plasma i.e., 5%, 10%, 15% and 20% were prepared in BWW medium supplemented with BSA and SFMF (s). The washed spermatozoa were incubated at 37°C, PH 7.4 at their final concentrations of 35×10^7s/ml. The effects of different concentrations of seminal plasma i.e. 5%, 10%, 15% and 20% on pattern of motility, percentage motility, capacitation and acrosome reaction were studied at each hour of incubation according to the procedure as described earlier.[14]

Results and Discussion

The effect of different concentration of seminal plasma i.e. 5%,10%, 15% and 20% on percentage motility and on different stages of the acrosome reaction is shown in Figs. 1 and 2 respectively. As shown in Fig. 1, 20% of seminal plasma stimulated the percentage motility of spermatozoa for one hour of incubation.

However, spermatozoa incubated in 5%, 10% and 15% seminal plasma showed a progressive decline in percentage motility with increasing time of incubation. The decline in percentage motility was more in 20% seminal plasma followed by 15%, 10%, 5% and 0% concentrations. Baas *et al.*[1] have also reported that the percentage motility in reactivated spermatozoa was enhanced to a greater extent immediately after the addition of higher concentrations of seminal plasma i.e. 20%, but was retained for a shorter period as compared to lower concentrations of seminal plasma i.e. 2%, 5% and 10% Baas *et al.*[1] have also reported that motility stimulating factors (s) are present in the low molecular weight fraction of seminal plasma and the damaging effect was confined to the high molecular weight, non-dializable fraction.

Increasing concentrations of buffalo seminal plasma also increased the circular motility and decreased the forward motility of spermatozoa. According to Dott *et al.*[8], seminal plasma was detrimental to the survival of ram spermatozoa and it also affects the surface properties of epididymal spermatozoa i.e. agglutination, sticking to the glass and eosinophilla. Addition of serum albumin delayed but did not prevent the detrimental effect of seminal plasma, although it had no effect itself on survival.

Higher concentrations of buffalo seminal plasma (15% and 20%) are more detrimental to spermatozoa. According to Baas *et al.*[1] the variable stimulation of motility obtained with BSA and the reduction in stimulation after dialysis of spermatozoa and the poor stimulation of motility of bull spermatozoa with BSA as reported by Harrison *et al.*[9] could be explained by a variation in the amount of low mol. wt. motility stimulating component retained by bovine spermatozoa after washing in Ficoll. The low molecular weight component is probably present in or bound to spermatozoa and is gradually lost during dilution and incubation with BSA until motility ceases. The low molecular weight factor has much in common with the "sperm motility factor"—extracted from washed epididymal spermatozoa.[2]

Our earlier studies have shown that the buffalo spermatozoa can be successfully capacitated in BWW medium supplemented with BSA and SFMF(s) at a sperm concentration of 35×10^7 s/ml, 7.4 pH, 5 mM Ca^{2+} and at 37°C[14]. The spermatozoa incubated at concentrations 5% and 10% started showing first stage of acrosome reaction i.e. swelling and thickening of acrosome after 2nd hour of incubation and showed an increase till 5th hour, thereafter, it declined progressively till 8th hour of incubation. While the spermatozoa incubated at 15% and 20% concentrations showed swelling and thickening of acrosome after 2nd and 3rd hour of incubation respectively, with a slight increase till 8th hour of incubation. The second stage of acrosome reaction i.e. vesiculation was observed after 3rd, 4th and 5th hour of incubation in 5%, 10% and 15% seminal plasma concentrations respectively and it showed an increase with increasing incubation time till 8th hour. The spermatozoa incubated at 20% seminal plasma concentrations did not show vesiculation. Shedding of acrosome was observed only in 5% seminal plasma concentration after 7th hour of incubation. But in the control sample i.e. spermatozoa incubated in BWW medium supplemented with BSA and SFMF(s) without seminal plasma started showing the three stages of acrosome reaction

after 2nd, 3rd and 6th hour of incubation respectively and the percentages of all the three stages of acrosome reaction were more in the absence than in the presence of seminal plasma, as is shown in Fig. 2.

According to Bedford,[3] decapacitation factors(s) bind to the sperm surface and marks sites of importance for the induction of acrosome reaction. The failure of buffalo spermatoeoa to complete acrosome reaction in the presence of higher concentration of seminal plasma may probably be due to the inhibition of vigour of motility and capacitation, which are prerequisite for acrosome reaction. Kanwar *et al*[11] have shown that in the presence of higher concentrations of seminal plasma, the human spermatozoa failed to attach and penetrate the zona pellucida. Decapacitation factors(s) may inhibit the acrosomal hydrolases, which resulted in the inhibition of fertilization.

Acknowledgement

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Gross Observations on Development of The Components of Caprine Conceptus (Capra hircus L)

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ABSTRACT

In all 330 gravid caprine genitalia in various stages of gestation collected from abattoir, were studied.

Gravid uterus, foetus and membranes from first (0.1 to 0.5 cm contour) to ninth (32.1 to 39.0 cm C.R.L.) Stage in single pregnancy weighed from 34.40 g to 3574.75 g. 0.29 g to 1757.38 g and 1.82 g to 365.55 g respectively and from second (0.51 to 2.0 cm C.R.L.) seventh (20.1 to 26.0 cm. C.R.L. stage in twin pregnancy weighed from 117.38 g to 3375.22 g 0.89 g to 561.70 and 14.29 to 484.75 g respectively.

Amniotic fluid increased from first to eighth (26.1 to 32.0 cm C.R.L.) stage and decreased in ninth stage. Allantoic fluid was greater upto fourth but amniotic fluid predominated thereafter upto eighth and later both were nearly equal.

Foetus as proportion of total conceptus increased from 0.82 to 49.16 percent from first to ninth stage while membranes and fluids increased initially but decreased from 19.24 to 10.22 and 44.49 to 17.87 percent respectively from fourth and fifth to ninth stage.

* * *

The enlightened interest in the goat as a farm livestock stems primarily from an appreciation of its economic viability due to high fertility and short generation interval which are essential qualities of milk and a meat producer. Only a few studies have incorporated growth and development of all components of conceptus in bovines and Ovines. However, there is dearth of information on development of caprine conceptus carrying single and twin foetuses at various stages of gestation. Such information can establish the study of the factors causing significant deviations from the normal develpmental patterns in the conceptus of goat.

Materials and Methods

In all 330 gravid caprine genitalia, 255 with single and 75 with twin foetuses in various stages of gestation, were collected from Nagpur Municipal abatroir, for this study.

After the separation of the foetal cotyledons to display the allantoic and amniotic sac, the fluid in each compartment was collected separately and measured. The foetal membranes were separated and weighed. Age of the conceptus (Stage of gestation) was assessed on the hasis of —

i) C.R. length as per the normograph on sheep foetuses perfected by Joubert (1956) and

ii) C.R. lengths and weights of foetuses as per

Harrison (1948) and Lyngset (1971) in goats and gestation groups were formulated as below:

Gestation group	Crown Rump Length (cm)	Gestation Period (days)	Number of Foetuses	
			Single	Twins
I	(contour) 0.60	upto 16	6	—
II	0.61 to 2.00	16 — 30	32	25
III	2.01 to 4.00	31 — 45	34	33
IV	4.01 to 9.00	46 — 60	64	34
V	0.01 to 14.00	61 — 75	46	26
VI	14.00 to 20.00	76 — 90	24	19
VII	20.01 to 26.00	91 — 105	20	13
VIII	26.01 to 32.00	106 — 120	17	—
IX	32.01 to 39.00	121 and above	13	—

According to the aforesaid gestation groups the parameters for foetus, membranes, fluids and gravid uterus were classified for single and twin pregnancy and statistically anlaysed.

Results and Discussion

Foetal Weight (Tabel 1)

Average weight of the foetus-embryo increased from 0.29 ± 0.02 g in first to 1757.38 ± 86.51 g in ninth stage in single pregnancy and from 0.89 ± 0.08 g in second to 561.70 ± 37.37 g in seventh stage of twin pregnancy. These observations are lesser as compared to those reported by Lyngset (1971) in goats. There was no significant difference in weight of the foetus in single and twins from third to fifth stage (45 to 90 days), although the weights in twins looked lesser it may be due to the lesser, C.R.L. values of foetuses in these groups. During sixth and seventh stages, the twins were 0.86 and 0.96 times those of singles in weight. These observations were in agreement with 0.92 ratio reported by Rattray *et al* (1974) during last 12 weeks of gestation in ewes. Similar observations were also reported by Koong *et al* (1975) and Robinson *et al* (1977) in ewes. Foetuses borne in right cornu weighed significantly greater in single pregnancy. Male foetuses were also significantly, heavier in single pregnancy. Male sex hormone with its anabolic effect might be hastening the prenatal development of male (Hafez, 1980).

Foetal Membranes:

Average weight of the membranes ranged from 1.82 ± 0.14 g in first to 365.55 ± 30.39 g in ninth stage in singles while from $14.29 \pm$ g in second to 484.73 ± 48.29 g in seventh stage in twin pregnancy. Membranes with right cornual pregnancy were significantly heavier in pregnancy with single foetus.

At ninth stage the weight of the foetal membranes was about 38 times than at second stage. It was higher than 27 times increase reported by Amoroso (1952) in sheep. In the present study, foetal membranes which were 13.26 times greater than the foetal weight at the end of second stage (2.0 C.R.L.) decreased to 0.21 times at ninth stage (39.0 C.R.L.) in single pregnancy. These observations are similar to those of Tiwari *et al* (1969) who reported 6 times higher foetal weight than that of membranes in goat at birth.

Foetal membranes increased in weight with that of foetus upto sixth (14.1 to

TABLE-1

AVERAGE WEIGHT OF FOETUS, FOETAL MEMBRANES, AMNIOTIC, ALLANTOIC AND TOTAL FLUIDS AT VARIOUS STAGES OF GESTATION IN SINGLE AND TWIN PREGNANCY OF GOATS

Stage of Gestation	Foetus (g)	Foetal membrances (g)	Amniotic fluid (g)	Allaotoic fluid (g)	Total weight of fluid (ml)
I Single Pregnancy					
I	0.29±00.02	0.82±00.14	0.60±00.10	3.25±00.65	4.17±00.38
II	0.72±00.05	9.55±00.78	2.52±00.30	19.40±01.53	22.27±01.59
III	2.99±00.22	32.20±02.94	20.66±02.32	43.02±04.11	63.16±05.66
IV	12.66±00.77	56.83±03.46	73.59±03.99	46.12±02.77	119.39±04.94
V	67.06±04.56	107.45±05.99	229.59±12.69	53.27±05.95	279.67±14.41
VI	208.78±15.96	221.36±21.04	370.27±13.58	104.22±08.63	459.03±22.13
VII	581.16±23.03	238.84±20.13	379.28±20.65	138.90±13.76	514.62±27.93
VIII	822.70±40.19	261.43±14.85	411.47±37.63	192.74±23.07	604.33±29.19
IX	1757.37±56.51	365.55±30.39	326.74±33.66	320.07±30.86	639.12±27.53
II Pregnancy (Twin Foetus)					
II	1.78±00.16	14.29±01.12	3.74±00.93	21.67±02.41	25.06±02.71
III	4.84±00.38	34.75±02.60	13.90±01.28	26.76±01.98	40.81±02.76
IV	23.66±01.88	79.49±04.23	68.60±06.36	58.29±08.47	129.83±13.44
V	118.16±09.46	222.52±16.55	183.56±09.23	75.35±11.02	258.31±10.30
VI	360.42±20.56	274.38±19.88	352.88±24.10	88.23±18.52	441.10±29.47
VII	11.50±35.42	484.73±39.29	395.58±39.05	124.98±13.57	520.56±44.36

TABLE — 2

COMPONENTS OF CONCEPTUS AND EMPTY UTERUS EXPRESSED AS PROPORTION (PER CENTAGE) OF WEIGHT OF GRAVID UTERUS AT VARIOUS STAGES OF GESTATION IN SINGLE AND TWIN PREGNANCY OF GOATS

Stage of gestation	C.R. Length (cm)	Foetus		Foetal Membranes		Foetal Fluids		Conceptus	
		Single	Twins	Single	Twins	Single	Twins	Single	Twins
I	0.01-0.5	0.82	—	2.31	—	11.78	—	14.91	—
II	0.51-2.0	0.92	1.52	12.29	12.17	28.67	43.04	41.88	56.39
III	2.01-4.0	1.66	2.88	17.92	16.40	35.15	38.52	54.73	57.20
IV	4.01-9.0	4.29	4.36	19.24	14.67	40.42	47.93	63.95	66.97
V	9.01-14.0	10.67	10.08	17.09	18.97	44.49	44.05	72.25	73.10
VI	14.01-20.0	16.90	18.50	17.93	14.01	37.18	45.28	72.01	77.87
VII	20.01-26.0	20.14	33.28	12.39	14.36	26.69	30.84	69.22	78.49
VIII	26.01-32.0	37.26	—	11.84	—	27.37	—	76.47	—
IX	32.01-39.0	49.16	—	10.22	—	17.87	—	77.26	—

20.0 C.R.L.) stage but remained more or less constant during later stages. Similar observations were reported by Elliot *et al* (1934) and Cloette (1939) and Barcraft (1946) in sheep.

A ratio of 1.55 to 2.02 was observed in weight of membranes of single and twin foetuses from sixth to ninth stage of gestation in the present study. These findings are in agreement with those of

Rattray *et al* (1974) who reported a ratio of 1.5 to 2.0 in ewes during corresponding period.

Foetal Fluids:

Amniotic fluid in single pregnancy increased from 0.60±0.10 ml. in first stage to 411.47±37.63 ml. in eighth stage and later decreased to 326.74±33.66 ml. in ninth stage of gestation. Allantoic fluid, however, increased gradualy from 3.35±0.65 ml. in first to 192.74 23.07 ml. in eighth stage followed by a boost to 320.07±30.86 ml. in the ninth stage.

Present observations on changes in proportion of allantoic and amniotic fluid upto (90 days) sixth stage are in agreement with those of Cloette (1939) who observed that the allantoic fluid accumulated slowly apart from an initial preponderence and that the amniotic fluid increased greatly so far as the first 90 days are concerned. However, in the present study the acceleration of allantoic fluid was observed at the end of eighth stage (120 days) instead of sixth stage (90 days). Present finding are in agreement with those of Bongso *et al* (1979) who observed these shifts at 5.5 and 31 cm C.R.L. Jamdar *et al* (1972) also reported excess volume of amniotic fluid from 70 to 140 days in local ewes and it was suggestive of the requirement of tropical environment. Mallor and Slater (1974) reported that in addition to the change in the distribution of foetal urine flow, factors which modify urine composition would be expected to alter indirectly the volume of amniotic fluid from at least 80 days until term.

Volume of total fluid rapidly increased from first to sixth stage whereafter the rate of increase slowed down. Similar observations were reported by Winter and Feuffel (1936) Malan and Curson (1937), Cloette (1939) and Arthur (1957) in sheep and Bongso *et al* (1979) in goats.

Components of Conceptue

(Table-2)

Weight of foetus as a percentage of the gravid uterus increased slowly upto fourth stage and rapidly thereafter from 10.67 in the fifth to 49.1 percent in the ninth stage of single pregnancy. Curson and Malan (1939), Rattray *et al* (1974) and Robinson *et al* (1977) also reported similar rapid increases in later stages.

Weight of the foetal membranes increased from 2.31 percent in the first to 19.24 percent in fourth stage (60 days) and then decreased to 10.22 percent by ninth stage in single pregnancy. Similar trend of initial increase upto about 75 days and reduction thereafter was observed in twin pregnancy also. Present findings are in agreement with those by Malan and Curson (1937) and Cloette (1939) in sheep. Wallace (1949) also reported the plateau values for placental weight around 90 days of gestation. Rattray *et al* (1974) reported the decrease in the proportion from 28.2 percent at day 70 to 7.4 percent at day 140 in Targhee ewes in single pregnancy. The proportion at 70 days, however, seem to be higher than about 19 per cent observed in the present study.

Foetal fluids increased from 11.78 percent in the first stage to 44.49 percent in fifth stage and then showed a slow decline to 17.87 percent at the end of ninth stage. Similar trend was observed in Twin pregnancy also. Rattray *et al* (1974) reported 37.3 and 19.3 percent values at 70 and 140 days respectively in sheep.

Although present study deals with the highly heterogeneous population, the

trend of observations is sufficiently marked and could thus be utilized for a better understanding of the components of conceptus in goats.

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Effect of Separation of Seminal Plasma on Quality of Frozen Buck Semen

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ABSTRACT

The percentage of motile sperm in semen frozen with and without seminal plasma was 48.31±3.11 and 52.38±3.06 respectively in egg yolk citrate fructose glycerol extender (EYCFG) and 63.25± 1.83 and 64.15±2.85 respectively in Tris egg yolk citric acid fructose glycerol extender (TEYCAFG). The corresponding values of damaged acrosomes (in percentage) were 15.97±1.87 and 21.77 ±2.47, and 10.73±1.16 and 15.27± 2.55. The percentage of motile sperm in semen frozen without seminal plasma was 56.00±1.50 and 47.50±3.55 in skim milk egg yolk fructose glycerol and raffinose egg yolk glycerol extenders respectively. The corresponding values of damaged acrosomes (in percentage) were 14.63±1.47 and 19.07±2.54. The split ejaculates processed with seminal plasma in latter two extenders were discarded before freezing due to poor sperm motility. The percentage of motile sperm did not vary significantly between semen processed with and without seminal plasma in EYCFG and TEYCAFG But the percentage of damaged acrosomes was significantly higher ($P<0.01$) in spermatozoa frozen without seminal plasma in EYCFG and TEYCAFG.

* * *

Removal of egg yolk coagulating enzyme by washing of semen was found to improve sperm survival during preservation at 4°-5°C in egg yolk citrate and Tris based extenders (Deka and Rao, 1984a). In the present study, the effect of separation of seminal plasma on quality of semen frozen in different extenders was studied.

Materials and Methods

Eight ejaculates consisting of 16 each from 5 local bucks were collected twice weekly using an artificial vagina during the period from April to June, 1983. The effect of separation of seminal plasma was studied in 4 extenders using 20 ejaculates (4 from each buck) per extender. The extenders used were egg yolk citrate fructose glycerol extender (EYCFG) (Mathew, 1974), Tris egg yolk citric acid fructose glycerol extender (TEYCAFG) (Hahn, 1972), skim milk egg yolk fructose glycerol extender (SMEYFG) (Rajkonwar *et al.*, 1977) and raffinose egg yolk glycerol extender (REYG) (Paggi, 1971). The extenders were prepared in two fractions. The fraction A was devoid of glycerol except in SMEYFG which contains 3 per cent glycerol. The pH of EYCFG, TEYCAFG and REYG was adjusted to 6.8. Immediately after collection, each ejaculate

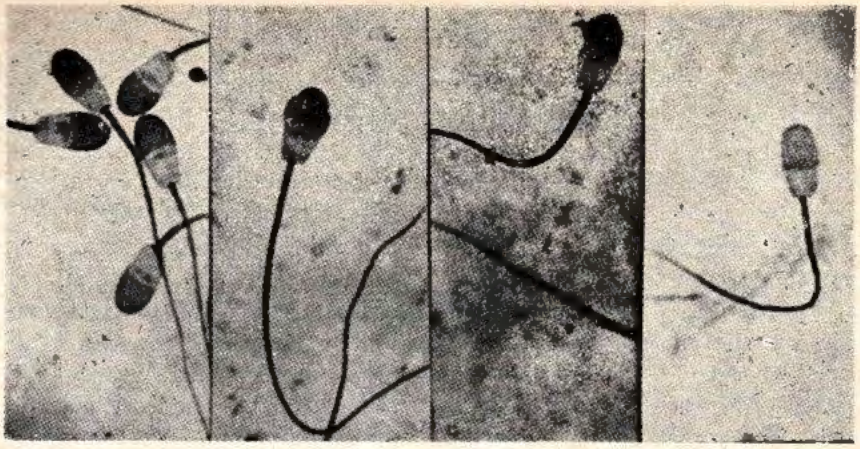

Fig. 1 Left to right: Sperm with normal acrosome, and sperm with swollen, separating and entirely lost acrosomes (damaged acrosomes).

TABLE 1. Percentage of motile sperm (Mean±SE) in semen processed with (P) and without (NP) seminal plasma in various extenders at different stages of processing of semen

Stages	Extenders					
	EYCFG*		TEYCAFG**		SMEYFG**	REYG**
	P	NP	P	NP	NP	NP
Fresh semen	86.00 ±0.96		86.95 ±0.56		83.95 ±0.74	86.60 ±0.50
After cooling to 5°C	78.44 ±1.76	80.63 ±1.14	79.10 ±1.58	83.60 ±1.02	79.75 ±1.08	79.25 ±1.45
After equilibration	75.50 ±1.89	76.44 ±1.57	76.10 ±1.85	78.10 ±1.42	74.45 ±1.23	75.35 ±1.89
After freezing	48.31 ±3.11	52.38 ±3.06	63.25 ±1.83	64.15 ±2.85	56.00 ±1.50	47.50 ±3.55

*Mean of 16 observations
**Mean of 20 observations

TABLE 2. Percentage of damaged acrosomes (Mean*+SE) in semen processed with (P) and without (NP) seminal plasma in various extenders at different stages of processing of semen

Stages	Extenders					
	EYCFG		TEYCAFG		SMEYFG**	REYG**
	P	NP	P	NP	NP	NP
Fresh semen	1.50 ±0.35		1.57 ±0.28		1.43 ±0.31	1.45 ±0.32
After cooling to 5°C	3.40 ±0.49	4.93 ±0.70	2.43 ±0.32	3.43 ±0.58	4.30 ±0.70	4.83 ±0.98
After equilibration	6.57 ±0.74	8.93 ±0.92	5.07 ±0.77	7.33 ±0.99	8.40 ±1.00	11.87 ±1.94
After freezing	15.97 ±1.87	21.77 ±2.47	10.73 ±1.16	15.27 ±2.35	14.63 ±1.47	19.07 ±2.54

*Mean of 15 observations

was split into two halves. One half was diluted @1:5 with fraction A of the extender. The other half was also diluted @1:5 with fraction A of the extender after removing the seminal plasma as described by Deka and Rao, 1984b. The initially diluted semen was gradually cooled from 35°C to 5°C in 1½ hours and fraction B of the extenders (amount equal to that of fraction A) was added to it at 5°C in 3 parts at an interval of 15 minutes. The freezing and thawing of semen were carried out as described by Deka and Rao (1984b). The percentage of motile sperm was estimated following conventional method. The incidence of damaged acrosomes was studied using Giemsa staining technique (Watson, 1975). The evaluation of semen in respect of percentages of motile sperm and damaged acrosomes was done at different stages of processing of semen viz., fresh semen, after primary dilution and cooling to 5°C, after equilibration and after 14 hours of freezing. The statistical analyses of the data were carried out after transforming the percentages into angles as per Snedecor and Cochran (1968).

Results and Discussion

The mean percentages of motile sperm and damaged acrosomes were presented in Tables 1 and 2.

The percentage of motile sperm did not vary significantly between semen processed with and without seminal plasma in EYCFG or TEYCAFG. This is in agreement with that of Corteel and Bril (1975). On the contrary, Westhuysen (1978) opined that removal of seminal plasma enhanced the post thawing motility of buck spermatozoa. In the present study the split ejaculates processed with seminal plasma in SMEYFG and REYG were discarded before freezing due to poor sperm motility (less than 40 per cent). But the split samples processed without seminal plasma maintained 56.00 and 47.50 per cent motile sperm in frozen semen in SMEYFG and REYG respectively (Table 1). Corteel (1974) also observed higher sperm motility before and after freezing in semen processed without seminal plasma in skim milk extender. Ont of 20 ejaculates studied, 4 split ejaculates processed with seminal plasma in EYCFG were discarded before freezing due to poor sperm motility. On the other hand, in case of TEYCAFG, no ejaculate processed with seminal plasma was discarded. The detrimental effect of egg yolk coagulating enzyme was found to be the highest with certain extenders, viz., SMEYFG and REYG and mild in other (EYCFG). In extender like TEYCAFG which is endowed with better buffering capacity the deleterious effect of the eneyme was the least. It is evident from this study that the deleterious effect of seminal plasma possibly depends on the buffering capacity of the extender used.

The different forms of damaged acrosomes are shown in Fig. 1. The percentage of damaged acrosomes was significantly higher ($P<0.01$) in spermatozoa frozen without seminal plasma in EYCFG and TEYCAFG. This might be due to the damage suffered by sperm during the process of separation of seminal plasma by centrifugation. This supports the observations of earlier workers on ram semen (Jones and Holt, 1974 and Marios, 1982). The percentages of motile sperm and damaged acrosomes varied significantly ($P<0.01$) between stages of processing of semen in all the extenders.

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Aerobic Bacteriology of Bovine Prepuce and Semen With and Without Prior Prepucial Washing in Jersey Bulls Maintained in Arid Zone

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ABSTRACT

4 clinically healthy Jersey bulls were used to isolate and identify the normal aerobic bacterial flora of the prepuce and semen.

The total bacterial load in the semen collected after prepucial washings was always lower than that collected without prior prepucial washings, except for a few occasions when the bacteria increased due to touching of penis of the bull to the hind quarters of the dummy before entry into the artificial vagina. *Bacillus, Corynebacterium, Streptococcus, Pseudomonas, Chromobacterium Micrococcus, Staphylococcus, Alkaligenes, Flavobacterium* and *Kurthia*, were isolated from the prepucial washings and all of them were also recorded in the semen.

Only *Kurthia*, which was isolated from prepucial washings of bull M-76, was absent in its semen.

* * *

Raghwan *et al.* (1971) isolated *Corynebacterium, Pseudomonas, Streptococcus, Bacillus, Escherichia* and Proteus in all the 9 prepucial and 8 semen samples, from apparently healthy bulls and buffalo bulls.

Meredith (1970) reported that the mean bacterial population per ml of ejaculate was 40.4×10^3 for 7 control bulls and 365×10^3 for 8 bulls with everted prepucial epithelium.

Meredith (1970) further studied the aerobic bacterial flora of ejaculates and semen straws from 15 bulls and reported the presence of *Chromobacteria, Corynebacteria, Escherichia coli, Proteus, Pseudomonas, Streptococcus* and *Staphylococcus.*

Brown *et al.* (1974) found that the total number of viable micro-organisms in semen from 42 bulls used for artificial breeding varied between 20 and 534000/ml with a mean of 4630 per ml.

Brown *et al.* (1974) investigated the semen of 42 bulls and found bacteria belonging to the genera, *Bacillus, Corynebacterium* and *Micrococcus, Aerobacter, Alkaligenes, Escherichia, Flavibacterium, Herella, Nocardia, Proteus* and *Sarcina.* These authors further concluded that the bacteria found in the semen were the transient invaders from urethra and prepucial cavity.

Materials and Methods

The present study was conducted from September, 77 to February, 1978 on 4 clinically healthy Jersey bulls (C-38, I-11, M-76 and CJ) stationed at the Department of Obstetrics and Gynaecology of the College of Veterinary and Animal Science, Bikaner for artificial insemination work. All the bulls were in the same

age group and were maintained under the same management condition.

Collection of samples:

Prepucial washings followed by semen samples were collected once a week on their normal collection days. During the following week, only the semen samples were collected without prior prepucial washing.

TABLE 1: Aerobic bacterial colony counts of prepucial washings in Jersey bulls

Bull No.	Range		Average
	Minimum	Maximum	
C—38	10.5×10^4	23×10^4	182588.33
I—11	16×10^3	11.3×10^3	58272.73
M—76	23×10^3	10.2×10^3	56583.33
CJ	22×10^3	12.0×10^3	45750.00

(i) *Prepucial samples:* After trimming the prepucial hair, the prepucial opening was washed with soap and plenty of water and dried with a clean sterile towel. 10 ml of sterile normal saline solution was infused into the prepuce, near the glans penis with the help of a sterilized glass Catheter attached to a 20 ml syringe. The sample was aspirated back after gentle up and down massage of the prepuce to mix the saline with prepucial smegma. It was then transferred aseptically to a sterile test tube, to which a cotton plug was applied.

On a few occasions prepucial opening was further cleaned with a spirit swab after washing with soap and water and then the prepucial washings were collected.

Semen was collected in the artificial vagina in the morning from individual bulls.

Total bacterial count: Total bacterial counts were made by pour plating with moltan sheep serum agar of serial dilutions 10^{-1} to 10^{-5} as per technique of Cruickshank (1965). After incubation at 37°C for 24 hours, plates were subjected to colony counts. Identification of bacterial isolates was done as per Cowan and Steel (1970).

Results and Discussion

The overall average of prepucial bacterial load of all the 4 Jersey bulls came out to be 84255.319 (Table 1)

It was observed that initially the bacterial load of prepuce was very high but with successive fortnightly washings with normal saline solution at the time of semen collections, it came down for all the bulls. This finding is in close agreement with that of Gunsalus *et al.* (1941)

TABLE 2: Bacterial isolates from the prepucial washings of Jersey bulls

Organisms isolated	Frequency of isolation/bull			
	C—38	I—11	M—76	CJ
Bacillus	2	3	5	11
Corynebacterium	12	7	2	10
Streptococcus	5	—	—	6
Pseudomonas	5	4	—	—
Chromobacterium	2	—	2	3
Micrococcus	3	4	4	1
Staphylococcus	—	4	—	—
Alkaligenes	—	3	5	—
Flavobacterium	—	—	1	2
Kurthia	—	—	3	—

TABLE 3: Aerobic bacterial colony counts of Jersey bulls semen.

Bull No.	With prior prepucial washings			Without prior prepucial washings		
	Minimum	Maximum	Average	Minimum	Maximum	Average
C—38	11×10^3	81×10^3	35791.67	7×10^3	87×10^3	48366.67
I—11	4.6×10^3	37×10^3	10583.64	13×10^3	62×10^3	31818.18
M—76	9×10^3	17×10^3	12833.33	11.9×10^3	34×10^3	16116.67
CJ	5.1×10^3	21×10^3	12975.00	12×10^3	41×10^3	19825.00

and Zemjanis (1962) who have observed a decline in prepucial bacterial flora with prepucial washings. However, in case of bull C-38 no appreciable decrease in the prepucial bacterial flora was recorded with successive washings and hence after washing the prepucial orifice with soap and water, it was cleaned with spirit swab and then collection was taken. This resulted in marked decrease in the total bacterial count.

The bacterial isolates from prepucial washings and the frequency of their isolation are given bullwise in Table 2.

A study of this table revealed 10 types of bacteria viz. *Basillus*, *Corynebacterium*, *Streptococcus*, *Chromobacterium*, *Micrococcus*, *Staphylococcus*, *Alkaligenes*, *Flavobacterium* and *Kurthia* recorded from the prepucial washings with a frequency of 109 isolates, out of which 29 were from bull C-38, 25 from bull I-11, 22 from bull M-76 and 33 from bull CJ. The isolation of *Chrombacterium*, Flavobacterium and Kurthia has not been reported earlier.

The overall average bacterial load for all the 4 bulls came out to be 29312.765 per ml in semen collected without prior prepucial washings and 18204.680/ml in semen collected after prepucial washings.

The range and average of the total aerobic bacterial colony counts with and without prior prepucial washings is given in table 3. By studying the table, it becomes clear that the total bacterial load in the semen was high initially but decreased with successive prepucial washings in all the bulls.

It was also noted that the bacterial load in semen without prior prepucial washings was always higher than the load with prior prepucial washings, except on few occasions where the bacterial load of semen with prior washings exceeded the load without prior washings. It was found that the glans penis of the bull had touched the hind quarters of dummy before entry into the artificia vagina.

The findings of a low bacterial load in semen collected after prepucial washings as compared to that collected without prior prepucial washings are in close agreement with Gunsalus *et al* (1941) and Zemjanis (1962).

The range and mean bacterial counts reported by Runceanu *et al.* (1976) and the mean bacterial counts given by Almquist *et al.* (1948), Easley *et al.* (1951), Meredith (197C) and Brown *et al.* (1974) are in close agreement with the present findings.

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Motility Index: A Simple and Reliable Test For Evaluating Semen Quality

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ABSTRACT

The superiority of the semen depends on the number of progressively motile spermatozoa present in it. Motility influences liveability and fertilizing ability. This is assessed by just visual examination and assigning a value based on an estimate will be more precise. The possibility of adopting a precise method of assessing the motility of spermatozoa has been suggested by using haemocytometer. The material consisted of fresh ejaculates from the breeding bulls which was examined microscopically after charging the haemocytometer chamber with semen sample of known dilution. The motility index was calculated by a formula derived and this was based on the forward motion of the spermatozoa and the coneentration of the sperms in the semen sample. It was observed that the motility index was significantly correlated with initial motility, live percentage, cold shock resistance test and liveability up to 48 hours in refrigerator. A table of correlation between all the semen characters studied revealed that the estimate of motility index is an easy and at the same time more precise test. This technique can be rapidly performed and does not require expensive equipments and at the same time more accurate.

* * *

The most prominent behaviour of the ejaculated spermatozoa is the unrelenting motility. Since its discovery, the motility of the sperm is being used as an important criteria for semen evaluation. Motility influences liveability and fertilizing ability. (Kruse, 1980). From time to time Scientists have evolved different methods for the assessment of motility of semen for andrological purposes. Some of the recent techniques are by using laser-light and fiber optic Dopler anemometer, laser-light scattering, computer monitoring, flourometric reflectometric techniques, motion picture films and spectrophotometric method (Ammann and Hammerstdt, 1980; Bar-Sagie *et al.*, 1981; O'Cannor *et al.*, 1981; Wool Ford and Harvey, 1982; Rose *et al.*, (1983). However most frequently employed technique is visnal observation and grading the motility. Accurate method of estimating motility requires sophisticated equipments. Visual observation is least expensive but accuracy and repeatability is questionable. These sophisticated equipments cannot be used for routine examination in all the semen processing laboratories. The present study was undertaken to evaluate a method to speed up the visual measurement of motility and make it more objective and accurate.

TABLE 1 Initial Attributes of Semen and Motility Index

	Volume (ML)	Mass Activity (0-5) after	Live Sperm-tozoa (Percent)	Live Sperma atozoa after cold shock (Percent)	Motility Visual Observa-vation (Percent)	Motility Visual Obser-vation after cold shock (Percent)	Motility Index after Dilution	Indexv After cold shock	Motility Index after 24 hours	Motility Index after 48 hours
Crossbred cattle	7.29± 2.18	2.59± 1.27	77.30± 7.99	22.66± 14.22	69.43± 9.84	21± 9.40	571.26± 345.03	131.62± 123.97	555.53± 296.14	340.91± 277.74
Buffaloes	5.27± 1.57	2.77± 0.87	84.33± 6.31	34.09± 6.95	71.42± 8.33	28·33± 9.60	892.93± 339.79	299.73± 125.41	442.55± 320.50	255.57± 183.65

Materials and Methods

Semen was collected from four crossbred and two Murrah buffalo bulls, which were routinely used for Artificial Insemination purpose in Southern Regional Station of National Dairy Research Institute, Bangalore. A total of fifty ejaculates were used in this study, which comprised of thirty five ejaculates from crossbred bulls and fifteen ejaculates from buffalo bulls. Immediately after collection of semen the volume, sperm concentration, mass-activity, live-dead count, motility percentage were recorded as per Herman and Madden (1972). Cold shock resistant test (Chinnaiya and Gangluli (1978) live and dead count and motility before and after cold shock test was also carried out. Semen was diluted with egg-yolk citrate and stored in the refrigerator and liveability was estimated after 24 and 48 hours of storage.

Motility Index was estimated as per the technique of Negai *et al.*, (1982), used in human semen which was modified by diluting the semen sample. Dilution rate was based on initial sperm concentration which was brought to the level of approximately ten million sperms per ml. This was found convenient and dilution was done with egg-yolk citrate extender. Haemocytometer was charged with diluted semen and microscopic examination was done using a biotherm. The number of spermatoeoa crossing on either sides of a 0.25 mm. line in any one of the white bood cell counting chambers per minute was counted. The counting was

TABLE 2 Correlation Coefficients Between Motility Index and other Parmeters

	Volume	Mass Activity	Motility (visual observation)	Live Spermatozoa (Percent)	Cold shock Resistant Sperms	Initial live Sperms and Motility Index After 24 hours	Initial live Sperms and Motility Index After 48 hours
Crossbreed Cattie	—0.19	0.18	0.18	0.55***	0.68***	0.56**	0.61***
Buffaloes	—0.15	0.30	0.43*	0.74***	0.67***	0.60**	0.59**

* ($P<0.05$)
** ($P<0.005$)
*** ($P<0.001$)

repeated four times in different lines of the chambers of the microscopic field and the average number of sperms crossing the line per minute was taken into account. Spermatozoa that were circling in the field were not considered. Motility Index was claculated by using the formula

$$\text{Motility Index} = \frac{\text{Number of spermatozoa crossing the line per minute.}}{\text{Number of spermatozoa per mili liter of diluted sample} \times 10^{*}} \times 10^{8}$$

The statistical analysis was carried out as per Snedecor and Cochran (1972).

Results and Discussion

The initial attributes of the semen quality inclusive of motility index are given in Table I and correlation coefficients obtained are shown in Table II. Highly significant correlation was observed between motility index and percentage of live spermatozca in the semen of cross-bred cattle and buffaloes ($P > 0.001$ Table II.) No significant correlation could be observed between Motility Index, volume, mass activity, and visual observation of motility in cattle semen which agrees with the reports of Louda and Smerha, (1981). Highly significant correlation was noticed between live spermatozoa in neat semen and motility index after 24 hours as well as 48 hours of storage at refrigeration temperature. Spermatoza surviving after cold shock were also correlated with motility index. In cross-bered cattle there was no significant change in motility index in neat semen and semen stored for 24 hours. But in buffalo semen a sharp decline was noticed after 24 hours of storage. The decline was not effectively exposed under visual observation in the present investigation. In earlier report also, Sexana *et al.*, (1978) did not find significant decrease of motility after 24 hours storage. This indicates that visual observation and grading the samples though simple, reliability is poor and repeatability is also lacking. Moreover in experiments involving continous monitoring of changes in motility visual observation and grading cannot be employed. In this study poor correlation was obtained between live spermotozoa and visual observation. Mann and Mann (1981) suggested to supplement visual observation of motility with live and dead staining to get better idea about quality of semen. Motility index is simple and elobarate procedures are not required which was successfully used in continuous monitoring of changes in motility by Negai *et al.*, (1982), in human semen. Moreover it is less expensive requiring only haemocytometer and repeatilbiity was high as could be seen from the results. In addition to this, motility index was highly correlated with live spermatozoa at all stages of estimation.

Before adopting motility index tests for large number of samples, a few factors wnich influence it to a great extent are to be considered. In this study the diution varied from 1:100 — 1:300 depending on the number of spermatozoa per ml. of the ejaculates. Process of dilution was extensive and to avoid the possible dilution effect, egg-yolk citrate was used which is an accepted extender and it successfully counteracted the dilution efiect. Newzealaud Dairy Board (1979) in in its production report mentioned the beneficial effects of egg yolk besides Ovalbumin and Globulins. According to Dott *et al.*, (1979) semen could be diluted up to 1:600 without any

significant decline in motility for a short period. Here also the Motility Index was estimated immediately after dilution to a convenient level so that the movement of individual spermatzoao was clearly visible.

Based on the above it may be observed that the method of estimating motility index can be used as a quantitative test for the routine evaluation of semen with better accuracy and uniformity.

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Studies on Estrus Synchronisation in Local Nondescript Heifers

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ABSTRACT

67 local nondescript heifers from three different contiguous centres of BAIF were selected for conducting trials in oestrus synchronisation. Silastic intravaginal coils (PRID), Norgestomet implants and prostaglandin $F_2\alpha$ were used for synchronising oestrus.

One hundred percent expression of heat was seen in group of heifers where PRID and implants were used while only 18 percent of heifers showed good standing heat and 77 percent showed silent heat in prostaglaudin group. In control group only two heifers showed heat within 21 days of withdrawal of treatment. The conception rate following treatment was 66, 50, 23 and 50 percent on first A.I. while overall conception rate within 90 days after withdrawal of treatment was 89.0, 50.0, 54.5 and 25.0 percent respectively in PRID, implant, PG and control groups. The conception rate and treatment to conception interval was significantly different as compared to control.

* * * *

It has been estimated that approximately 50% of estrus periods in dairy cattle in U.S.A. are not detected and that approximately 20% of cows presented for insemination were not in physiological estrus (Kiddy, 1978). This is much worse in India where farmers lack training in heat detection. The heats in local nondescript heifers are discrete, and go undetected. The age at maturity therefore varies greatly from 24 months to 48 months. It is of great interest to investigate the benefits of the treatments which will synchronise estrus and inseminations on predetermined dates.

Materials and Methods

A total of 67 nondescript heifers were selected for the study from three different places in operational area of BAIF Cattle Breeding Centres. They were alloted to three different treatments and control groups. The grouping was done according to the body weight as shown below:

All heifers were checked for ovarian cyclicity before being included in the trial. This was done by two rectal palpations at ten days interval. The group 1 was introduced with progesterone releasing intravaginal device (PRID — CEVA —FRANCE) The silastic coils. These

	Silastic coils	Implants	Prostaglandin	Control
Groups	1	2	3	4
Number	9	20	22	16
Body Wt. Kg.	214±26	207±52	197±18	206±24

TABLE 1. Heifers detected in oestrus 2-4 days after treatment compared to control animals.

Group Oestrus	Silastic Coils (n = 9)	Implants (n = 20)	PGF2α (n = 22)	Control (1) (n = 16)
Standing (2)	9	20	4	1
Silent (3)	—	—	17	1
Absent	—	—	1	14

1. Heifers seen in heat in the 21 days following the end of the treatment period.
2. Standing oestrus in estrus clearly seen by farmers.
3. Silent estrus in estrus not reported by the farmers but checked and confirmed by rectal palpation.

were iserted in the vagina for 10 days. This coil contained additionally one capsule of 10 mg. of oestradial benzoate. At the time of removal 300 i.u. of pregnant mare serum gonadotrophin (PMSG) was injected intramuscularly. The heifers were inseminated at fixed times viz. 48 and 72 hours after the removal of coil. These heifers were also checked for visual heat symptoms. In group 2 the heifers were treated with an implant containing 6 mg. of Norgestomet (Intervet France 17 acetoxy-11 B methyl-19 nor preg 4 ene 3.20 dion) the implants were placed subcutaneously on the outer portion of the ear for 10 days. At the time of implanting 6 mg. Oestradial valerate plus 3 mg. of norgestomet was injected intramuscularly. At the time of removal of implant 300 i.u. of PMSG was given intramuscularl. As per the previous group checking of estrus and insemination performed at 48 and 72 hours after removal of implants. Animals of group 3 were treated with two injections of 25 mg. of prostaglandin $F_2\alpha$ No estrus detection was done after 1st injection but checking of heats and insemination performed on a fixed time basis at 72 and 96 hours after last injection. The cotrol heifers were injected with 5 ml. saline on day 1 corresponding to 1st day treatment in the 3 treated groups. Heat detection examination twice a day and insemination on observed heat started on the 11th day after inclusion on the trial.

All inseminations were done with frozen semen produced at BAIF Uruli-Kanchan with known average fertility. All heifers were followed for subsequent ninety days and were reinseminated if repeated. All the animals were diagnosed for pregnancy by rectal palpation 60 days after last insemination. Chisquare test was carried out as per Snedecor. and Cochran (1950) to test the significance.

Results and Discussion

Expression of Oestrus: All the heifers in the treatment group 1 and 2 showed one hundred precent heat at 48 to 72 hours after withrawal of the treatment while in group 3 ($PGF_2\alpha$) only four heifers showed standing heat (18 percent) while 17 heifers (77 percent) showed silent heat and one heifer did not show heat at all. In control group 2 heifers only showed heat one standing and one silent (Table 1.)

TABLE 2. Conception rate at estrus following synchronisation treatment Percentage

Silastic coils (n = 9)	Implants* (n=20)	PGF2* (n=22)	Controls* (n=16)	Mean (n=67)
66	50	23	50 (1/1)	33

* Only estrus detected animals were taken into account.

TABLE 3. Percentage of heifers pregnant by 90 days after treatment and mean intervals between treatment and conception.

Group Fecendity	Silastic Coils	Implants	PGF2α	Control
% of pregnant animals bred before 90 days	89.0*	50.0*	54.5*	25.0
Mean interval treatment to pregnancy (days+SD)	21±18*	12±0*	26.5±22.1*	43.0±14.0

* Significant at 5% level.

Conception Rate: The heifers which had showed heat symptoms were only considered for calculating conception rate. The conception rate was maximum in group 1 (66% followed by group 2 (50%) and group 3 (23.0%) while in the control group only two heifers showed heat and only one heifer conceived. The heifers which did not concieve in first A.I. were inseminated as and when they expressed heat. The three successive heats were considered and the period was restricted to 90 days after treatment In this period 89.0, 50.0 54.5 and 25.0 percent conception rates were obtained respectively in group 1, 2, 3 and 4. (Table 2 and 3).

All the treatment groups had shown significantly higher conception rate as compared to control. The mean interval from treatment to conception was 21±18, 12.0±0, 26.5±22.1 and 43.0±4.0 days respectively for group 1,2,3 and 4. These intervals were significantly lower as compared to control. (Table 3).

Although no comparable data under similar conditions involving all the three treatments reported herein is available in literature yet attempts with single treatments have been reported by different workers. Abeyratne *et al* (1943) used $PGF_2\alpha$ (Cloprostenol) for synchronizing 134 local Shrilanka heifers and reported that 19 percent of the treated heifers showed marked heat while 52 percent moderate and 28 percent poor or nil. These findings of Abeyratne *et al* (1983) are in total agreements with tne findings presented in this paper. In tne present study 18 percent of treated heifers showed marked heat symptoms which could not be missed by the farmers, while 77 percent of the treated heifers showed silent heat which in 4 percent of the treated heifers the heat was absent. The conception rate reported by Abeyratne *et al* (1983) in heifers also matched with the conception rate reported in the present study.

The results obtained in the present

study of synchronising oestrus in heifers by the use of PRID or Norgestomet subcutenous implants are in complete accordance to the findings reported by Kher *et al* (1982); Beghelli *et al* (1982) who had used PRID for synchronising oestrus in Hostein Friesian cows and heifers and beef cattle and Fuenmoyor, *et al* (1982) who had used Norgestomet subcuteneous implants for synchronising cows.

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Listeria Infection in Anestrus Cows and Buffaloes: A Serological Assessment

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ABSTRACT

178 serum samples from anestrous cows and buffaloes were subjected to tube agglutination test against *L monocytogenes* serotype 4b as per Osebald and Aalund (1968). The sera samples showing a titre of 1:50 or more were taken as positive for listeriosis 33 (25%) out of 131 farm animals and 7 (15%) out of 47 field animals revealed listeria agglutinin positive titres and indicated the possibility of prevalence of listeria infection in farm as well as field animals. Necessity for a check for listeriosis in problem animals, is therefore suggested.

* * * *

Listeria monocytogenes infection in reproductive tract of cattle is well recorded in the different parts of the world (Purkucin *et al.*, 1970; Gayon, 1972; Sixl *et al.*, 1978; Gitter, 1979 and Guillox, 1979). In India Dutta and Malik (1978) and Srivastava *et al.* (1982) reported an isolation of *L monocytogenes* 4b from stomach contents of aborted fetus and serotype ½C and 4b from uterus of infertile cows respectively. Limited information on listeriosis appear to be available in the

TABLE 1 Particulars of animals from which the serum samples were tested

	Cow-heifers	Cows	Buff.-heifers	Buffaloes	Total
Farm animals	81	33	9	8	131
Field animals	3	14	4	26	47
Total	84	47	13	34	178

TABLE 2 Serum titres of listeria antibodies in anestrus cows and buffaloes

	Serum dilutions					Total No. positive
	1:50	1:100	1:200	1:400	1:800	
Farm animals (131)	1	13	8	5	6	33 (25.19%)
Field animals (47)	0	3	3	0	1	7 (14.89%)
Total (178)	1	16	11	5	7	40 (22.47%)

TABLE 3 Group particulars of listeria agglutinin positive animals

	Cow-heifers	Cows	Buff-heifers	Buffaloes	Total
Farm animals	14	12	3	4	33
Field animals	—	—	2	5	7
Total	14	12	5	9	40

literature on problem cows and buffaloes. The present study aimed at the serological assessment of listeriosis in anestrus cows and buffaloes.

Materials and Methods

178 serum samples from anestrus cows and buffaloes from organised farms and field area were subjected to tube agglutination test against *L monocytogenes* (serotype 4b) as per Osebald and Aalund (1968). The particulars of the animals from which the serum samples were examined are presented in Table 1.

Results and Discussion

Out of 178 serum samples, 40 sera samples showed listeria agglutinin positive titre of 1:50 or more. These animals were regarded to be positive for listeria infection. The various serum titres observed in the animals studied are presented in Table 2.

The group particulars of the animals showing listeria agglutinin positive serum titres are presented in Table 3.

The observations in the present study indicate the presence of listeriosis in the anesturs cows and buffaloes in the area. It appears therefore necessary to consider and take account of listeriosis also while dealing with the problem breeding animals.

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FARM NEWS

Cross Breeding Work in Amul Area

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ABSTRACT

Although it is difficult to cover all the female crossbred born under field condition upto their production age due to socioeconomic condition of farmers and some administrative problem, field data available indicates that irrespective of type of cross-breds, they are performing well under field condition. The over-all performance of these cross-breds indicates that age at first calving is 2 years 6 months and 15 days (1213 samples) lactational milk yield in 300 days—2608.7 liters with 287 lactation days (2504 samples), general fat percentage 4.1% (917 samples) and intercalving period as 14 months 10 days (1377 samples) respectively. Further their furture productivity can be sustained with aforesaid planned breeding and exploiting the feeds and fodder resources at village level.

* * * *

Eventhough various animal husbandry input programmes for a couple of decades were tried to increase the milk production in surti buffaloes, the effect was quite marginal. The average increase was only from 3.2 ltrs to 4.5 ltrs. Therefore, if doubling the milk production is aimed at, it would take another 60-70 years. It was, therefore, decided to cross-breed the local cows to exploit the effect of hetrosis and effect an immediate increase in fluid milk production. From 1973-74 onwards, Amul decided to accept cow milk as a part of initiation of introducing cross breeding programme in Kaira district. The in-built resistance to change and interest in selling only bullocks and not milk by the community who keeps cows in the distirict slowed down the pace of progress.

Amul's Venture

A. There are about 58,000 cows (Kankrej, Gir and non-descript breed) in the district. Initially the cows from North Gujarat were brought and distributed to the progressive farmers to start cross breeding work. In addition, cross-bred cows and heifers purchased from Military Farms were distributed in the district. The corss-bred pregnant heifers from Haryana were also purchased and distributed to boost-up the work.

B. *Selection of breeding bulls and breeding programme:* The Jersey sires born out of proven sires and maintained at Institute of Agriculture, Anand and S.A.G.P., Bidaj Farm, both managed by IDC/ NDDB were used for cross breeding work in the district.

In the month of December 1976, Amul launched 'Amul Heifer Project' wherein pure-bred Holstein—Friesian Heifers

TABLE 1 Number of inseminations in cows and the number of cross-bred born in operational area of Amul.

Year	No. of A.I. performed		No. of Cross-bred born		
	Fresh	Total	Female	Male	Total
1974-75	1956	2582	67	55	122
1975-76	3208	4127	270	246	516
1976-77	3568	4326	316	298	614
1977-78	4729	5128	467	416	883
1978-79	6334	7667	520	496	1016
1979-80	7792	10620	736	639	1375
1980-81	9307	11756	799	722	1521
1981-82	8507	11812	873	698	1571
1982-83	10513	16383	1746	1239	2985
1983-84	12048	20119	1796	1560	3356
Total:	67962	94520	7590	6369	13959

TABLE 2 Monthwise Insemination in cows and cross-bred born from 1974-75 to 1983-84.

Month	No. of Insemination	% to the total insemination	No. of crossbred born	% to the total CB born
April	6883	7.3%	1130	8.1%
May	6856	7.3%	1070	7.7%
June	7595	8.0%	942	6.8%
July	8369	8.8%	823	5.9%
August	7617	8.0%	1052	7.5%
September	7641	8.1%	1347	9.6%
October	7894	8.3%	1208	8.6%
November	8082	8.5%	1216	8.7%
December	8665	9.3%	1219	9.2%
January	8869	9.4%	1365	9.8%
February	7812	8.2%	1203	8.6%
March	8237	8.8%	1308	9.5%
Totrl:	94520	100%	13959	100%

(between 3-6 months of age) were imported from Canada and reared them in the holding farm till maturity and calving. The semen of outstanding progeny tested bulls were imported from Canada to get these heifers pregnant. The male calves born out of this stock were also reared for the future cross breeding programme in the district.

With a view to maintain blood level in between 50% to 75% the young Jersey and H.F. cross-bred bulls with 50% blood level born out of high yielding local cows were purchased and reared upto their breeding age at Sperm Station of ARDA.

C. The semen from the pure-bred and cross-bred bulls maintained at the farm is being frozen and preserved to be used in villages where the cow population is

more than 50. In each of these villages lay inseminators were trained in the use of frozen semen insemination technique. To boost-up the work at village levels attractive incentives in kind and cash are given both to the inseminator as well as to the owner of the cow.

Provision has also been made to make available the desired exotic half bred cow bull semen to the interested farmers on demand at all the veterinary sub-centres where a farmer may come along thermos flask with ice to collect with it in order to inseminate his cow in heat through the local worker who is working daily for the insemination of buffaloes at the village society level.

In case of emergency where the cross breeding facilities are not available the farmer may avail the services of mobile veterinary unit by calling a special visit for insemination at a nominal cost of Rs. 15/-. The insemination in special visit is carried out by trained veterinarians at farmers, door on demand.

The attempts of the Amul towards cross breeding programme in last decade can be very well appreciated vide Table No. 1.

The total insemination in cows from inception of cross-bred scheme were also classified monthwise to study the effect of season if any on breeding and calving of cross-bred calves. Monthwise break up of insemination and its percentage to the total insemination and monthwise crsoss-bred calves born and its percentage to the total calving are shown in the Table No.2. This table indicates that the lowest number of inseminations in cows were performed in the month of April and May while it was highest in the month of December and January However, the differenee was 2.05% which indicates that there was not much impact of season as far breeding of cows are concerned. Similarly the highest calving was recorded in the month of January—9.8% while it was lowest in the month of July (5.9%).

Recording System

As indicated above, the cross breeding work has been taken in selected villages and utmost care has been taken to maintain and record the progress of cross breeding both at village and Union level. Individual pedigree records are maintained in duplicate (one at owner's place and other at Unions' office) so that every animal can be followed up.

The economical production performance of Jersey, H.F. and tripple crosses are studied as regards to their (i) Age at first calving (ii) Milk Yield in 300 days and lactation days (iii) Full lactational yield and full lactation days (iv) General fat percentage (v) Dry days (vi) Gestation period and (vii) Inter-calving period.

The table No. 3, 4 & 5 indicate the production performance records of Jersey, H.F. and tripple crosses respectively.

The over-all productivity performance of cross breds is shown in the Table No. 6.

Observation

It is quite clear from Table No. 6 that the age at first calving is minimum in the case of triple crosses 2 yrs. 4 months and 27 days (95 samples) when compared with its contemporaries. Jersey crosses 2 yrs 6 months and 10 days (976 samples) and Holstein Crosses 2 yrs 9 months and 7 days (142 samples) respectively but the number of first calving studied for tripple crosses is too less when compared with Jersey crosses.

As far lactational milk yield in 300 days is concerned table No. 6 indicates that

TABLE 3 Production Performance of Jersey Cross-breds (Age at first calving—2 years, 6 months & 10 days (976 samples)

Lactation No.	Lactational Milk yield in ltrs. (300 days)	Lactation days	Full lactational yield (ltrs.)	Full lactation days	fat percentage (%)	Dry days	Gestation period Months, days	Inter-calving period Months Days
1	2345.0 (989)	287 d. (989)	2922.5 (610)	365 d. (610)	4.1% (428)	114 d. (769)	9M. 3d. (911)	Not applicable
2	2502.0 (571)	286 d. (571)	3179.7 (302)	368 d. (302)	4.1% (232)	101 d. (434)	9M. 2d. (271)	14M. 13d. (597)
3	2798.0 (267)	289 d. (267)	3477.4 (154)	366 d. (154)	3.9% (82)	94 d. (202)	9M. 2d. (150)	13M. 25 d. (249)
4	2835.4 (82)	288 d. (82)	3342.7 (48)	363 d. (48)	4.0% (28)	83 d. (60)	9M. 5d. (60)	13M. 29d. (97)
5	2825.0 (23)	287 d. (23)	3233.0 (10)	349 d. (10)	3.5% (3)	66 d. (15)	9M. 2d. (12)	12M. 13 d. (23)
6	2779.8 (5)	280 d. (5)	3760.5 (2)	354 d. (2)	—	126 d. (3)	9M. 6d. (5)	12M. 24 d. (7)
OVER-ALL	2481.3 (1937)	287 d. (1937)	3089.5 (1126)	366 d. (1126)	4.1% (773)	106 d. (1483)	9M. 3d. (1409)	14m. 5 d. (1018)

M = Months, d = days. Figure in brackets indicates number of lactation studied for that particular parameter.

TABLE 4 Production Performance of H.F. Cross-breds (Age at first calving—2 years, 9 months & 7 days (142 samples)

Lactation No.	Lactational Milk yield in ltrs. (300 days)	Lactation days	Full lactational yield (ltrs)	Full lactation days	fat per-centage (%)	Dry days	Gestation period Months, days	Inter-calving period Months, days
1	2808.3 (145)	290 d. (145)	3683.0 (98)	385 d. (98)	3.9% (42)	101 d. (122)	9M. 4d. (133)	Not Applicable
2	3059.9 (112)	286 d. (112)	4234.6 (66)	405 d. (66)	3.7% (27)	117 d. (85)	9M. 4d. (54)	14 M. 25 d. (118)
3	3354.3 (74)	287 d. (74)	4310.0 (51)	400 d. (51)	3.7% (21)	102 d. (63)	9M. 4d. (51)	14 M. 24 d. (82)
4	3618.1 (44)	296 d. (44)	4363.8 (33)	375 d. (33)	3.7% (10)	117 d. (33)	9M. 3d. (32)	15 M. 22 d. (52)
5	3187.6 (22)	288 d. (22)	4327.8 (15)	402 d. (15)	3.2% (4)	89 d. (14)	9M. 9 d. (16)	13 M. 14 d. (24)
6	3383.4 (8)	270 d. (8)	3986.6 (4)	342 d. (4)	3.6% (1)	62 d. (3)	9M. 8 d. (4)	12 M. 25 d. (9)
OVER-ALL	3097.6 (405)	289 d. (405)	4064.0 (267)	392 d. (267)	3.8% (105)	106 d. (320)	9M. 4 d. (291)	14 M. 24 d. (285)

M = Months, d = days. Figure in the brackets shows number of lactation studied for that particular trait.

TABLE 5 Production Performance of Tripple Cross-breds (Age at first calving—2 years, 4 months & 27 days (95 samples)

Lactation No.	Lactational Milk yield in ltrs. (300 days)	Lactation days	Full lactational yield (ltrs.)	Full lactation days	fat per-centage (%)	Dry days	Gestation period Months days	Inter-calving period Months, days
1	2739.2 (95)	295 d. (95)	3237.2 (72)	392 d. (72)	3.8% (25)	111 d. (65)	9M. 1 d. (96)	Not applicable
2	3021.7 (45)	288 d. (45)	3508.3 (27)	374 d. (27)	3.5% (7)	101 d. (29)	9M. 2 d. (18)	15 M. 5 d. (49)
3	3412.5 (19)	288 d. (19)	3688.0 (13)	349 d. (13)	3.8% (8)	100 d. (10)	9M. 4 d. (11)	13 M. 7 d. (21)
4	2670.0 (2)	300 d. (2)	2747.5 (2)	309 d. (2)	4.1% (2)	49 d. (1)	9M. 0 d. (2)	16 M. 7 d. (3)
5	3993.0 (1)	300 d. (1)	4309.0 (1)	352 d. (1)	3.8% (1)	—	—	12 M. 27 d. (1)
OVER-ALL	2903.5 (162)	292 d. (162)	3352.8 (115)	381 d. (115)	3.8% (39)	107 d. (105)	9M. 2 d. (127)	14 M. 21 d. (74)

M = Months, d = days. Figure in the brackets shows number of lactation studied for the particular trait.

TABLE 6 Comparative production performance of three types of cross-bred cows

Sr. No.	Type of cross-bred	Age at first calving	Lactation Milk yield in ltrs. (300 days)	Lactation days	Full lactation yield in ltrs.	Full lactation days	fat per-centage (%)	Dry days	Gestation period Months Days	Inter-calving period Months Days
1.	Jersey cross-bred	2Y.6m.10d. (976)	2481.3 (1937)	287 d. (1937)	3089.5 (1126)	366 d. (1126)	4.1% (773)	106 d. (1483)	9M. 3d. (1409)	14M. 25d. (1018)
2.	H.F. cross-bred	2y.9m.7d. (142)	3097.6 (405)	289 d. (405)	4064.0 (267)	392 d. (267)	3.8% (105)	106 d. (320)	9M. 4d. (295)	14M. 25d. (285)
3.	Tripple cross-bred	2y.4m.27d. (95)	2903.S (162)	292 d. (162)	3352.8 (115)	381 d. (115)	3.8% (39)	107d. (105)	9M.2d. (127)	14M.21d. (74)
OVER-ALL PERFORMANCE		2y.6m.15d. (1213)	2608.7 (2504)	287 d. (2504)	3282.2 (1508)	371 d. (1508)	4.1% (917)	106d. (1908)	9M.3d. (1831)	14M-10d. (1377)

Y = year, M = months, Figure in the brackets shows number of lactation used for that trait.

it is highest for H.F. crosses 3097.6 litres (405 samples) in 289 days while tripple crosses remained in 2nd position and yielded 2903.5 litres (162 samples) in 292 days. Although the Jersey crossbreds yielded 2481.3 litres (1937 samples) in 287 days but the number of lactation studied were too high when compared with other two crosses. Almost same trend remained when full lactational milk yield and lactation days were studied for these three different types of cross-breds from Table No. 6.

The fat percentage in milk of these three different types of cross-breds were also studied and given in table No 6. It was recorded highest in case of Jersey cross-breds 4.1% (773 samples) while it was 3.8% for H.F. cross-breds (105 samples) and tripple crossbreds (39 samples). The dry days remained practically same, 106 days, for Jersey cross-breds (1483 samples), H.F. cross-breds (320 samples and tripple crossbreds (105 samples). Similarly no apparent difference in the gestation period of these three different types of crosses were observed and was recorded as 9 months, 3 days for Jersey crossbreds (1409 samples), 9 months 4 days for H.F. crossbreds (295 sampes) and 9 months 2 days for tripple crossbreds (127 samples) respectively. It is also quite evident from the table N. 6 that the inter-calving period for Jersey, H.F. and tripple cross-breds were recorded as 14 months 5 days (1018 samples) 14 months 25 days (285 samples) and 14 months 21 days (174 samples) respectively.

Although the 300 days and full lactational milk yield was recorded highest in case of Holstein Cross-bred cows, farmers' choice remained for Jersey cross-bred cows. The probable reasons for this choice seems to be better adaptability of Jersey cross-breds in the local condition and its higher fat content(%) in the milk which fetches attractive price, through local milk coops. More over their male calves can be raised better and used as bullocks under field conditions.

Future

Recent conversion of liquid semen centres into frozen semen centres has made it possible to maintain the desired exotic blood level in the cross-bred population upto some extent. As per the recommendation, blood level of the cross-breds will be maintained between 50-75% by the restricted use of both types of exotic semen (Jersey and H.F bulls) in pure indigenous stock (desi cows) only while extensive use of ½bred bulls will be made in rest of the female cross-bred population. These half cross-bred bulls will be selected initially from the high yielding desi cows of the operational area and will be introduced in the field. The performance of these bulls will be judged by their daughters performance under field condition. Thus, after testing these bulls, the above average bulls will be used extensively to produce furture breeding bulls in the area subjected to their daughters performance test.

ISSAR NEWS

1. Congratulations to Prof. C.R. Sane

Our patorn Prof. C.R. Sane has been unanimously elected as the Vice-Chairman of the Indian Association of Fertility & Sterility. IAFS is the All India Body formed by eminent human gynaecologists. Prof. Sane is instrumental in bringing together, the human and animal gynaecologists on one forum and his unanimous election as Vice-Chairman IAFS vindicates the contribution of Veterinarians in Research in Reproduction.

2. NOTIFICATION

FIRST ASIAN CONGRESS ON ANIMAL REPRODUCTION DEC. 1985.

The ISSAR in association with Konkan Agricultural University, Dapoli and the Indian Council of Agriculture Research, New Delhi, proposes to organise the First Asian Congress on Animal Reproduction during December-1985 at Bombay. Eminent Scientists from Japan, Philippines, Thailand, Malaysia, Indonesia, Pakistan, Balngladesh, Sri-Lanka, UAR-Egypt and Iraq would be invited to participate in the deliberations of the congress.

ISSAR is likely to announce the final dates by April-1985. Agricultural Universities, State Departments of Animal Husbandry, National Institutes, Dairy Federations, Pharmaceuticals and other industries are requested to depute their representatives to participate in the Asian Congress 1985. All concerned are requested to contribute papers.

(Dr. D. P. Velhanker)
Hon. Secretary, I.S.S.A.R.

3. NOTIFICATION

Nils Lagerlof Memorial Award-1984.

The Indian Society for the Study of Animal Reproduction is pleased to invite research clinical articles on the subject of Animal Reproduction published by Indian Authors in any of the journals during January to December-1984, for consideration of the Nils Lagerlof Memorial Award for the year 1984.

Four copies of the reprints of the articles 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 Hon. Secretary, ISSAR, latest by 31st. May-1985.

The awards for 1983 and 1984 are proposed to be presented at the inaugural function of the First Asian Congress on Animal Reproduction to be held at Bombay in December-1985.

(Dr. D.P. Velhankar)
Hon. Secretary, I.S.S.A.R.

4. List of Life Members of ISSAR (Continued)

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Bombay-400 012

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
The Indian Journal of Animal Reproduction
The Indian Society for the Study of Animal Reproduction

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Information on the main program will be made available shortly. For further details, contact:

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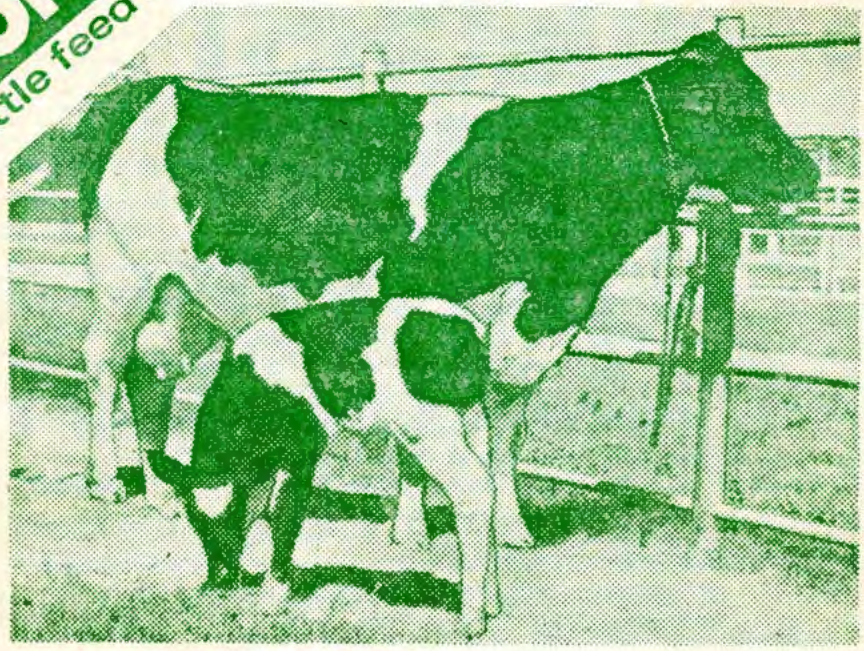

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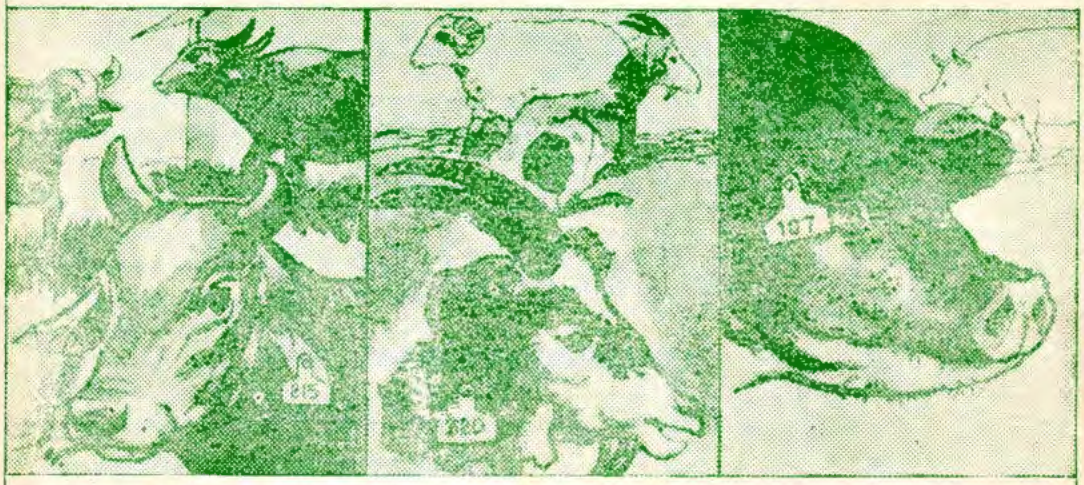

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