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Optimization of Reproduction in Dairy Animals

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Research Article

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ABSTRACT

Reproductive performance is one of the important factors for determining the economics of livestock production. Factors leading to sub fertility or infertility can broadly be classified as hereditary, physiological, nutritional, manage mental, environmental, pathological, psychological, immunological incompatibility and immune-deficiency syndromes. Keeping these factors in mind, a multifaceted research project was planned out under various subtitles as under, constituting 1000 cross bred cows and Murrah buffaloes , 50 exotic cross bred goats from NDRI and CIRB institutes of ICAR, few thousands rural buffaloes and cows in 32 villages around Karnal- India, 30 male sheep from the college of veterinary and animal sciences Bikaner, India and 100 buffalo genital organs from abattoir, Barely(UP) for the biometry at the college of veterinary and animal science Pantnagar.

Biometry of buffalo genitalia: The first step to be taken to ensure the enhancement of reproductive efficiency was to do biometry of the buffalo genitalia in order to assess the necessary operations involved during artificial breeding. The study suggested that the measurements of cervix, corpus uterus, uterine horns, fallopian tubes and ovaries of 100 adult buffaloes indicated that the organs were slightly smaller than in foreign breeds of cows. The average length of left ovaries was found to be 2.44 cm, width1.34 cm, thickness 1.58 cm, weight 3.32 gm. and of right ovaries as 2.36 cm, 1.37 cm, 1.64 cm, weight 3.13 gm, respectively[1].

Antigenic studies of RAM (male sheep) semen subjected to auto immunization.

Electrophoreses of sperm and seminal plasma antigens in male sheep subjected to auto immunization.

Induction and synchronization of estrus correction of reproductive disorders in farm and rural dairy animals.

Monitor the process of follicular and luteal development using ovarian sonogram, laparoscope, surgical (laparotomy) and nonsurgical procedures.

Collection and preservation of male & female germ plasma, super ovulation, embryo collection and transfer (ETT), IVM, IVF and cloning.

Estimations of Macro-Micro minerals during anestrum, repeat breeding, puerperal and post-partum phases of reproduction.

Induction of parturition, early pregnancy diagnosis (EPD) based on progesterone estimations in blood plasma and milk by RIA.

Monitoring of endocrine profiles including prostaglandin F2α, FSH, LH, estradiol-17β, progesterone, cortisol and prolactin during various phases of post birth, growth, puberty, maturation, reproductive cycle (follicular & ovulation), puerperal partum, postpartum, and pregnancy.

Effects of different season body weight and weaning on the endocrine profiles in female buffalo calves.

The recent techniques and strategies for the use of sexed germ plasm, stem cells, automated heat detection devices, sex determination by lacer detection techniques,(LTD), and feeding of somatotropin (bST) hormone are some more which needs to be incorporated in such programs. However, use of genomics, proteomics and bioinformatics will undoubtedly provide researchers with a greater understanding to enhance efficient reproductive process in animals. The other emerging assisted parameters will be the incorporation of zoo noses and public health aspect.

INTRODUCTION

Reproductive performance is one of the important factors for determining the economics of livestock production. Factors leading to sub fertility or infertility can broadly be classified as hereditary, physiological, nutritional, manage mental, environmental, pathological, psychological, immunological incompatibility and immune-deficiency syndromes. Keeping these factors in mind, a multifaceted research project was planned out, constituting 1000 cross bred cows, Murrah buffaloes, 50 exotic cross bred goats from NDRI and CIRB institutes of ICAR, few thousands rural buffaloes and cows in 32 villages around Karnal-India, 30 male sheep from the college of veterinary and animal sciences Bikaner, India and 100 buffalo genital organs from abattoir, Barely (UP) for the biometry at the college of veterinary and animal science Pantnagar.

I feel privileged to be the pioneer in estimating the Prostaglandin F2α cross bred cows, buffaloes and goats in India during growth and various phases of reproduction. Established modern facilities to measure most of hormones e.g. Follicle Stimulating Hormone(FSH), Luteinizing Hormone(LH), Prolactin (PRL), Growth Hormone(GH), Progesterone(P4), Estradiol-17β(E2), Cortisol and Gastrin by Radioimmunology Assay (RIA) with and without extraction of blood plasma and milk.

Reproductive disorders and postpartum fertility status among dairy animals

Prevalence of puerperal and postpartum reproductive disorders among crosses of Holstein Friesian and Zebu (KF 756), Brownswiss and Zebu (KS 677), Sahiwal (145) and Murrah Buffaloes (492) were investigated for a period of three years at NDRI Karnal India and were found to be 38.1, 26.7, 15.9 and 15.7% in KS, KF, Sahiwal and Murrah buffaloes, respectively. The prenatal losses in the form of abortions and still births were also lower among Zebu animals including buffaloes. However, Murrah buffaloes had higher rate (2.6%) of difficult parturitions in comparison to cross bred cows. There by it was difficult to put forward any hypothesis for being having a higher rate of reproductive disorders in crosses of KS in comparison to KF [2].

Biometry of buffalo genitalia

The first step to be taken to ensure the enhancement of reproductive efficiency was to do biometry of the buffalo genitalia in order to assess the necessary operations involved during artificial breeding and to monitor all events after fertilization. The study suggested that the measurements of cervix, corpus uterus, uterine horns, fallopian tubes and ovaries of 100 adult buffaloes indicated that the organs were slightly smaller than in foreign breeds of cows. The average length of left ovaries was found to be 2.44 cm, width1.34 cm, thickness 1.58 cm, weight 3.32 gm and of right ovaries as 2.36 cm, 1.37 cm, 1.64 cm, weight 3.13 gm, respectively [1].

Induction of estrus in postpartum dairy animals

Routine periodical examination after 30 days partum, douching with Lugols iodine (10-20 ml, 0.5%), two applications per weak, administration of estrus inducing drugs e.g. Prajana, Uteroline, Fertivet, Secrodyl, Folligon/PMSG and Prostaglandin F2α(lutalyze) were tried in cases without showing genital infection. Whereas animals showing genital infections were subjected to the application of intra uterine(I/U) lugols solution 10-20 ml (0.5%), two applications on alternate days, and in some cases oxytocin 50 IU I/m after the last application of lugols solution, I/U administration of three injections on alternate days of pancreomycin 20 lakhs (20 ml) or terramycin(10 ml), or gentamycin(2-5 ml) or two injections of metrijet (Intervet) and finally PGF2α 25 mg- two injections at 12 days apart. The responses of PGF2α over other drugs to induce estrus synchronization were very encouraging in livestock in general. It was also tried in few hundred rural, farm bred and cross bred cows, buffaloes and goats responding

within 96 hr of PGF2α treatment [3]. Since the losses due to the death of early embryo were very heavy and perhaps the principle factor for lowering the reproductive efficiency in cows, buffaloes and goats, these animals showing such problems were further subjected to screening by ultra sound, endoscopy (in some cases), laparotomy, tubal patency, and mineral profiles by atomic spectrometer and reproductive hormonal profiles by RIA.

Induction of postpartum ovarian response in buffaloes

Post-partum (75) buffalo cows were subjected for induction of estrus with Gn-Rh-200 µg (n=8), Lutalyse-25 mg I/m (n=26), Folligon-500 IU (n=11), 750 IU (n=14), and 1000 IU (n=8), and Synchromate-B ear implants (n=8). The overall ovarian activity in the treated groups was then compared with untreated buffaloes(n=60) The response of estrum exhibition was found to be 37.5, 50.0, 18.0, 63.0, 50.0, 25.0, and 26.7 % among Gn-Rh, Lutalyse, all groups of Folligon, Synchromate- B and control groups, respectively [4].

Postpartum endometritis in dairy animals

Intrauterine therapy constituting different drugs was tried in cases of postpartum endometritis in Karan Swiss (123), Karan Friesian (91), Sahiwal (7) cows and buffaloes (23). The drugs used were Metrijet (n=17), Terramycin liquid (n=53), Betadine (n=22), Veteran LA (n=81) and Furea (n=7), which took on an average of 16, 18, 20, 18, and 19 days for declaring clinically cured, respectively with a no significant difference among all treated groups in cases of postpartum endometritis [5].

Comparative efficacy of estrus inducing drugs in rural dairy animals

The overall incidences of reproductive disorders in rural dairy animals (2843) were observed very low in comparison to farm bred animals. Non cyclic breed able dairy animals(1712) were subjected to eight treatments namely ovarian massage, lugols iodine paint on cervix, Herbogyne capsules, Cocu tablets with lugols paint, enucleation of CL, mineral mixture with lugols paint, vitamins A & D3 and mineral mixture alone resulted in induction of estrus as 40, 51, 52, 47, 79, 47, 62, and 44%, respectively. However a divergent trend in conception rate was observed in animals treated with mineral mixture with lugols paint(70), Herbogyne capsules(60), lugols paint on cervix(57), cocu tablets(56),Vitamin A & D3(54), enucleation of CL(33) and mineral mixture(27%) [6].

Comparative efficacy of drugs to reduce the occurrence of repeat breeding in dairy animals

To minimize the incidence various drugs were administered in 329 repeat breeding dairy animals showing pathological and morphological genital conditions. These repeat breeding animals were further sub divided in to two groups, as animals showing a mild degree of infection (group 1) and in (group 2) animals showing sub clinical and nonspecific pathological conditions. These animals were given separately I/U liquid terramycin solution, Mast alone–U, Benzene penicillin G sodium, and lugols solution of 0.5%. The other group of 224 animals having no history of genital infections was given P4, HCG, and oxytocin I/m during routine AI. The response in both the groups was recorded on the basis of conception within 42 days of administration (2 estrous cycles). The response of 0.05% lugols iodine solution was observed most effective (67%) followed by liquid terramycin and benzyle penicillin G sodium (52-53%) and Mast alone-U (41%). Whereas the response in second group was most effective with 125 mg P4(68%), followed by HCG (65%) and oxytocin (64%). The study suggested that the incidence in repeat breeding dairy animals with mild infection can be minimized with the application of lugols iodine solution and animals with no infection with P4 and HCG. However, further trials are needed to substantiate these findings [7].

Tubal potency test

Tubal patency test by performing laparatomy was tried in some repeat breeding animals, not conceived even after treatment and four inseminations. A mild solution of blue dye (50 ml) was injected in the utero-tubal junction by using a 50 ml syringe. In all such cases the dye passed through the entire fallopian tube and reached to the ovarian fimbria and to the abdominal cavity ensuring no blockage in the oviduct. This experiment was done just to ensure whether the failure of conception is because of some blockages in the fallopian tube of the animals.

Early pregnancy diagnosis(EPD) on the basis of milk and blood plasma P4 levels

Milk P4 levels to monitor early pregnancy in goats and buffaloes was estimated by a direct RIA without extraction and purification. Milk samples (20 ml) each on days 19, 21, 25, and 27 post mating were collected from goats. The P4 concentration in milk was found to be significantly higher (P>0.01) in pregnant goats with a peak level of 39.49 ± 3.23 ng/ml on day of mid gestation. The accuracy of the test was 100 %(13/13, 10/10, 6/6, 4/4) by taking a level of 7.25 ng/ml or above as an indication of pregnancy[8]. Similar trials with almost same results in buffalo's milk were reported [9].

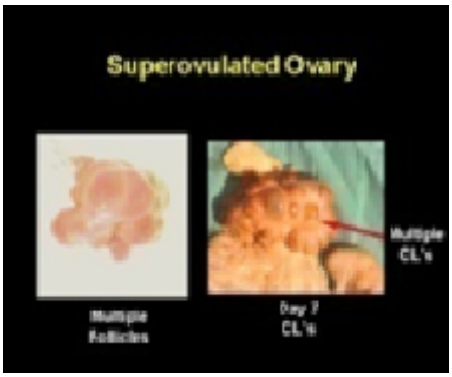
Induction of parturition in goats

Eight pregnant goats were subjected to administration of PGF2α (dinoprost-20 mg) and dexamethazone (Dexona 20 mg) separately for termination of pregnancy 10 days before the expected date of kidding. The control goats (n=4) were not subjected

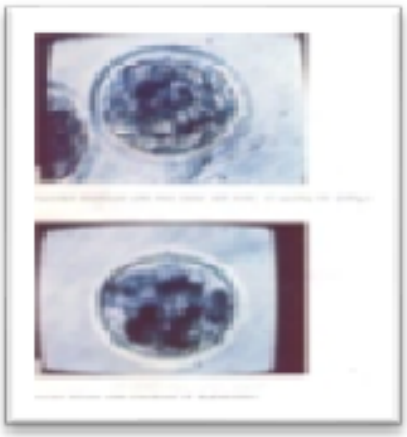
to any treatment. All induced goats had normal kidding with healthy kids. The goats induced with PGF2α and dexamethazone took 35.3 hr and 54.66 hr, respectively for the delivery after administration. Placenta was dropped within 2to5 hr of kidding, both in control and induced group. The mean corresponding body weight of male and female kids were 3.22 kgs and no significant difference was observed [10].

Embryo transfer technology in dairy animals

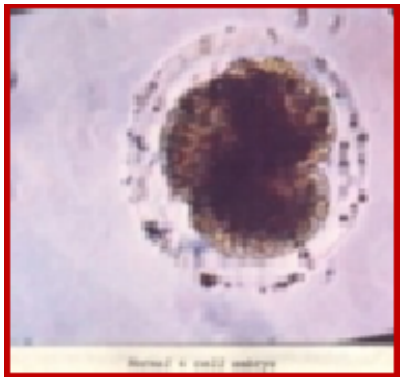
Murrah buffaloes were monitored for ovarian activity and estrus expression for studies on super ovulation and embryo transfer project during the period from 1988 to 1994 by administrating different super-ovulatory regimen with FSH-P, FSH-E, PMSG, OVAGEN, FOLLITROPIN and SUPEROV (n=256) in various combinations and different pattern of doses. Out of these 133 buffaloes were flushed non-surgically on day 5, 6, and 7 of first insemination **Figures.1-8.** In addition 24 nonsuper ovulated normal cycling buffaloes were also flushed during this period. The expression of estrus in donors and recipients were observed within 48-60 and 60-72 hr, respectively after PGF2α administration. The donors were inseminated three times after the onset of estrus. The overall ovarian response in terms of follicles and corpora lutea was observed as 0.3 and 2.03, respectively with a maximum response in Follitropin induced group(n=35)[11-14].



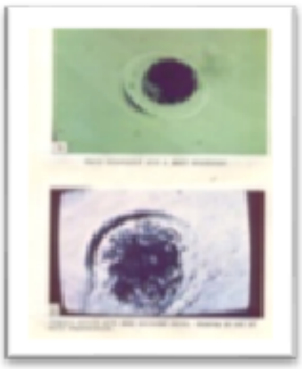
Figures 1. Super-ovulation and embryo evaluation.



Figures 2. Super-ovulation and embryo evaluation.



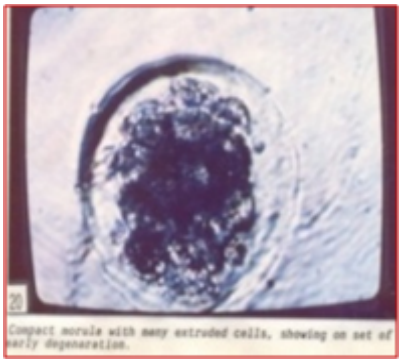
Figures 3. Super-ovulation and embryo evaluation.



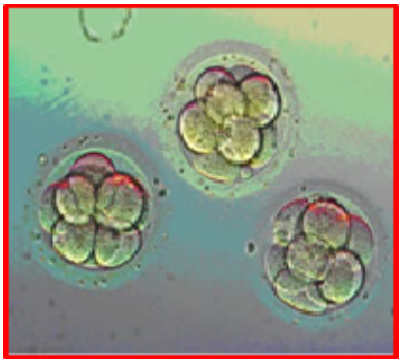
Figures 4. Super-ovulation and embryo evaluation.

Figures 5. Super-ovulation and embryo evaluation.

Figures 6. Super-ovulation and embryo evaluation.



Figures 7. Super-ovulation and embryo evaluation.



Figures 8. Super-ovulation and embryo evaluation.

Ultrasonography of follicular and corpora luteal development in buffaloes and goats subjected to super ovulation

Trans rectal ultrasonography was employed once daily before and after 8 hr administration of FSH (follitropin 30 mg) in 12 buffaloes for screening the total number of follicles and their developmental stages. Buffaloes with 2 or more corpora lutea were considered as responders. The number of medium and large sized follicles and the total number of follicles were significantly different ($P<0.05$) between responders and no responders while no difference was observed in small follicles. The study indicated that the pool of prenatal follicles is responsible for the development of large follicles destined to ovulate. The analysis of correlation coefficient between large sized follicles and corpora lutea was positive($r=0.47$). This technique was helpful in ascertaining the actual number of CL and pattern of follicular development during luteal phase of estrous cycle [15]. Similar trials were done in goats subjected to super ovulation with PMSG, HCG and PGF2α.The response was studied by performing laparotomy(surgically) and measuring the size of follicles in mm [16].

Efficacy of different collection methods for oocyte retrieval in buffaloes, goats and sheep

Three different methods were employed for oocyte retrieval from ovaries collected from a nearby abattoir in buffaloes. Ovaries were subjected to procedures of slicing (N=131), follicle puncture (n=86) and follicle aspiration (n=80). The slicing technique yielded not only significantly ($P<0.01$) more (5.7) oocytes per ovary as compared to follicle puncture (2.6) and aspiration (1.7) but also good quality oocytes. However, the time taken in slicing and aspiration techniques was more in comparison to other technique. Similar observations were noticed when the ovaries from sheep were subjected to slicing technique for oocyte retrieval [17-19]. However, the technique of follicular puncture was employed to collect the ovaries of goats (n=347) as the collected oocytes were used for maturation process for IVF studies [20].

Monitoring of macro and micro mineral profiles in buffalo heifers associated with fertile and nonfertile inseminations. Circulatory levels of Calcium(Ca), iron(Fe), zinc(Zn), copper(Cu), manganese(Mn), and cobalt(Co) were estimated by atomic absorption spectrometer in buffalo heifers following breeding. The levels were on an average 48.42 ± 1.27, 1.04 ± 0.05, 0.54 ± 0.02, 0.76 ± 0.02, and 0.01 ± 0.00 µg/ml in fertile heifers which settled to insemination and 46.31 ± 1.06, 1.01 ± 0.06, 0.65 ± 0.04, 0.76 ± 0.09, and 0.02 ± 0.00 µg/ml which did not settle after insemination, respectively. However, the levels of Zn, on days 1, 3, 17, 21 were lower significantly ($p<0.5$) in conceived heifers [21].

The levels associated with puerperal and postpartum problems were also measured in cross bred cows of Fe, Zn, Cu, Mn and magnesium(Mg) during the period of 40 days postpartum in normal and cows having had puerperal problems of abortions, still birth and retained fetal membranes. The average levels of Fe, Zn, Mn, Co, and Mg on the day of parturition in normal calvers were 3.2 ± 0.9, 0.8 ± 0.1, 1.3 ± 0.4, 2.7 ± 2.1 and 63.5 ± 6.4 µg/ml, respectively with higher levels of Fe and Cu on the day of parturition in normal calvers in comparison to cows having had reproductive problems. Whereas the levels were lower in normal calvers for Zn and Mn without any variations in the levels of Mg among normal and abnormal calvers. However, the levels of Zn, Fe, Mn and Cu were found lower in cows retaining fetal membrane. The study suggested that the level of trace minerals have some role to play in retaining the fetal membrane in cross bred cows [22].The levels associated with anestrum and repeat breeding problems in bovines were also measured in blood plasma in twelve Murrah type rural buffaloes, five repeat breeding buffaloes and four cross bred cows for various mineral profiles during a period of 28 days at a weekly interval in anestrum buffaloes and on days 1,3,6,10,13,16,19,22 and 25 in repeat breeding cows and buffaloes.

The average levels of Mn, Cu, Mn, Zn, and Fe were 1.66, 0.98, 0.65, 1.45, and 1.68 µg/ml, respectively in anestrum rural buffaloes. Whereas the overall levels were 52.33, 1.34, 0.50, 0.88, 1.64 and 52.1, 0.99, 0.52, 0.96, and 2.1 µg/ml, respectively in repeat breeding buffaloes and cross bred cows. The levels of Cu and Zn were found lower in repeat breeding buffaloes and of Cu, Mn, Zn and Fe in repeat breeding cows, respectively on the day of breeding (day-1) in comparison to other days of estrous cycle. The study suggested that the lower concentration of trace minerals on the day of estrus in repeat breeding buffaloes and cows have some role in failure of conception and needs more trials.

In vitro maturation (IVM) and fertilization (IVF)

Attempts were made to fertilize the unfertilized ovum collected during the routine non-surgical flushing and collection of embryos in buffaloes. The media used for oocyte maturation and embryo development was T CM-199+10% buffalo estrus serum. These ova were challenged with a heavy dose of high quality semen and were incubated in BO media having 20 mg/ml BSA and 10 µg/ml heparin for 3 hr and then sperm attached oocytes were transferred to maturation media drops for further development. The maturation media was changed on alternate days and the oocytes were observed for cleavage under phase contrast microscope and resulting embryos were cultured for nine days [20]. However, now enough progress has been made in culture conditions, such that immature oocytes can now be retrieved and matured *in vitro* (IVM).

Cloning

It is an asexual form of reproduction where all the genes of the offspring would come from a cell of a single individual: Attempts were made to produce clones from some buffalo blastomeres for the multiplication of superior germ plasm at a faster

rate during routine work of embryo transfer. The blastomeres were aspirated by puncture technique using a very fine needle fitted with a sterilized syringe. The collected blastomeres were then incubated as per the technique used for IVF.

Calves born from embryo transfer technology in buffaloes

In all 122 embryos were recovered out of which 72 were transferred into 68 synchronous recipients. The recipients were examined for pregnancy status on day 55 to 60 of embryo transfer and so far 15 buffaloes were declared pregnant(9/36 in FSH-P and 6/28 Follitropin group) withal normal calves(4 males and 7 females). The body weight of male and female calves at birth ranged between29-35 and 22-26 Kgs. with a gestation period of 313 and 305 days, respectively. However, no significant difference in ovulatory responses was observed in buffaloes super ovulated with FSH-P, ovagen, and folligon [23].

Hematological profiles during peri and postpartum periods in crossbred cows

Hemoglobin (Hb), Packed Cell Volume (PCV) and Differential Leucocytes Count(DLC) were studied up to 40 days of parturition in 24 crossbred dairy cows, which had problems of abortions(6) and still birth(5). The Hb content(g%)was found lower in cows which had aborted(10.3 ± 1.1) and thrown still born calves(10.2 ± 0.6) on the day of calving in comparisons to normal calvers(11.0 ± 0.4) The values of PCV%(34.0+_4.5 ± and lymphocytes%(48.1 ± 5.3) were lower and of neutrophils(41.7 ± 5.6%) and eosinophils (3.0 ± 1%) were higher in aborted cows on the day of parturition in comparison to normal calvers having had an average levels of PCV 39.9 ± 1.4, lymphocytes 62.1 ± 2.9, and neutrophils 26.8 ± 2.4 and eosinophils 1.8 ± 0.6%. The haematological indices did not fluctuate significantly during the postpartum period from day one to day 40 of parturition in both categories of cows. However, the levels of PCV (45.6%) and Hb (11.8%) were higher in KS than in KF cows (PCV 42.5% and Hb10.6%). It was also observed that cows having abortions and still births invariably suffered from retention of placenta following metritis (72.8%), whereas this incidence in normally parturated cows was 18% only, which needs further investigation[24].

Semen collection, evaluation and freezing of buffalo bulls, crossbred bulls and Rams (male sheep)

Electro ejaculation, Surgical, and Artificial Vagina (AV) techniques were employed for the collection of semen in different species of animals during the research conducted under report. Mostly electro ejaculation method was used in male sheep [25] and Artificial Vagina method in other animals [26]. Whereas surgical method was used only when the semen was to be collected from rate testis in bulls. The collected semen was examined for volume, color, density, mass activity, pH, sperm concentration and abnormal sperms percentage. It was clear from the results that there was no deterioration in semen volume and sperm concentration during the experimental period when the semen was collected daily for 2-3 weeks (11-25 days). A better freezability of buffalo semen using 6% glycerol level was observed during routine freezing trials [27].

Effect of auto immunization of semen and testicular homogenate on semen quality and testes histology

Chokla rams(n=10) kept in three groups as control(n=2), Immunization 1(n=6) and immunization 11(n=2) were immunized s/cut with Fruend's aduvant at an interval of three days with semen and testicular homogenate for a period of one month. The rams in immunization group I was given the last injection after seven days and in group II after 15 days of the sixth injection. The immunized rams in both the groups showed a remarkable decrease in sperm concentration and an increase in the abnormal sperm count. The histological examination of the testes showed, in general, edema, fibrosis on interstitial tissue, distention of seminiferous tubules, degeneration of Sertoli and Spermatogenic cells, thickening of the basement membrane and hypo spermatogenesis. However, rams kept in the control group did not show any change at any level except some vacuolization of Sertoli cells in one ram [25-27].

Antigenic analysis of spermatozoa and seminal plasma by Gel diffusion and Immuno electrophoresis in Rams

The double Gel diffusion and immune electrophoresis of the sperms and seminal plasma antigens of different breeds of Rams(exotic & local) revealed different seminal antigen pattern in rams and reflects the genetic difference among the different breeds of the same species. Immunological finger prints can therefore be obtained of the ram semen for the first time, which could be of great importance in breeding practices [28].

Electrophoresis of semen and blood serum proteins of rams

Agar gel electrophoresis separation of proteins and spermatozoa, seminal plasma and blood serum were carried out in Russian Merino, Rambouillet and Chokla rams (6 each) A total of six protein components(3 major and 3 minor) in seminal plasma protein and four (2major and 2 minor) in spermatozoa proteins were observed. The albumin values in the seminal plasma and spermatozoa were very low than in blood serum. Whereas the globulin content of the proteins of spermatozoa was much lower than the seminal and blood serum proteins in rams [29].

Monitoring of prostaglandin F2α and hormones profile from birth to puberty

Progesterone: Buffalo female calves were screened for P4 profiles from birth to day 3(n=67) showed a mean concentration

of 0.27 ± 0.07 ng/ml. The levels fluctuated in between035 ± 0.14 to 0.67 ± 0.08 ng /ml up to 30 months of age. However, the levels gradually increased to 0.8 ± 0.10 (n=29) after 30 month in animals showing cyclicity which then increased to a level of 1.18 ± 0.15 (n=14) in pregnant heifers. The variations in the different age groups were found highly significant(P<0.01,n = 404). and was also found significant when the analysis of variance was applied to study the effect of season, body weight, weaned and unweaned groups of calves [30].

Estradiol-17β

The levels fluctuated between 6.8 pg/ml to 12.8 pg/ml during the first 12 months of age and then decreased from days 181 to 270 to a lowest level of 4.5 ± 2.6 pg/ml (P>0.05) at the age of 361-450 days. The levels then slowly increased during the pubertal life and a level of 11.76 pg/ml brought the heifers to cyclicity [30].

Luteinizing hormone

The levels in the new born calves (days1-3) were significantly higher (P>0.01) than those in 1-30 days of age. The LH levels gradually decreased. However, no significance difference in the levels was observed in cyclic and non-cyclic heifers exceeding 30 months. However, the levels fluctuated from 1.09 ± 0.07ng/ml (n=53) on days 1-3 of age to 0.57 ± 0.14 ng/ml (n=15) on 3-6 months and then rose gradually up to months 9-12(0.74 ± 0.10 ng/ml, n=27). However, the overall mean levels did not differ significantly in cyclic and non-cyclic heifer [30].

Prostaglandin F2α

A marked difference in PGF2α concentrations was observed in the suckling and no suckling buffalo calves and being higher in non-suckled calves as in [31-33]. However, no definite pattern in the levels were observed when monitored among different age groups, body weight, weaning calves, and seasons but a slight increase in levels were observed during hot humid months [34].

Cortisol hormone

The cortical hormone profiles were measured by a direct RIA without extraction of plasma. The plasma cortisol levels among neonates were found high immediately after birth (19.60 ± 0.61 ng/ml (n=19) and significantly declined subsequently post birth. The mean levels fluctuated between2.31 and 1.06 nag/ml among calves post nasally between 15 days and 180 days, 2.24 and 3.73 ng/ml among prepubertal and between 4.51 and 7.19 ng/ml among peripubertal heifers. However, no significant difference in the levels was observed between pregnant (up to day 22) and non-pregnant [33].

Hormone profiles during estrus and early pregnancy in buffalo heifers

Progesterone: The mean concentration of progesterone was maximum (0.72 ± 0.22 ng/ml) on 3 days before estrus and then decreased to a minimum of 0.22 ± 0.03ng/ml(P<.0.01)) on day one of estrus exhibition at around 09.00 hr(around ovulation) and increased slightly on day 2 after estrus, indicating the beginning of the formation of CL as in [34].The levels in the pregnant animals gradually increased significantly(<0.01) till day 22 post insemination as in [34].

Estradiol-17β

The mean plasma estradiol concentration ranged between 3.8 and 30.4 pg/ml(P<0.05) during estrus and ovulation, with a major peak on day 0 at 07.00 hr and two other peaks of 22.5 and 21.4 pg/ml on day + 1 at 15.00 hr and 07 hr, followed by another three minor peaks of 15.4, 14.8 and15.5 pg/ml on days + 1, +2 and 0 at 03.00, 09.00 and 0.09 hr,respectively as in[34]. However, the levels declined from day 1 to 11 in non-pregnant heifers without much variation in pregnant heifers [35].

Luteinizing hormone

The LH levels in all animals greatly fluctuated (P<0.05) among days and time intervals of collections and the peak was observed on different days of estrus in all animals. Two animals showed peals of 5.0 ng/ml and 39.7 ng/ml on Day+0ne (ovulation day), whereas a peak of 62.9 ng/ml was observed on day_one of estrus (preovulatory day) in one buffalo. However, the overall LH levels did not differ significantly between pregnant and non-pregnant heifers [34].

Prostaglandin F2α

The mean levels of PGF2α ranged between 0.05 and 1.75 ng.ml from day 2 before estrus to day 3 after estrus, with a significant difference (p<0.05) from day +3, with +1 at 05.00 hr of estrus. However, all major peaks were shown at the end of estrus and before ovulation on day +1 at 15.00 hr and 17.00 hr as in [34].Day to day comparison of PGF2α levels between pregnant buffaloes showed that only days 13, 22, 16 and 7 were having significantly higher (P<0.05) and (P<0.01) PGF2α on first day 5 of estrus and day 16 to other days in non-pregnant heifers [34].

Cortisol Hormone

No significant difference in the cortisol levels were observed between pregnant and non-pregnant heifers sampled post insemination up to day 22 [33].

Ovulatory changes and hormone profiles of PGF2α, LH, Estrdiol17-β and progesterone in buffaloes

The present study was under taken to find out the temporal relationship amount the peripheral blood plasma levels of PGF2α, LH, Estradiol17-β and progesterone during ovulation. Three pluriparous lactating buffaloes were selected and blood samples were taken daily on days -5, -4, -3, -2, -1, +2 and +3 of estrus and four hourly during the first 16 hr. of estrus and two hourly during the remainder of estrus and ovulation. The PGF2α levels increased significantly (P<0.05) from day 1 at 05.00hr-day 3 whereas the LH levels in all animals fluctuated widely (P<0.05) among days and time intervals of collection, with peaks on day+1 (ovulation day). The estrogen peaks were observed on day 0 at 07.00 hr. followed by another three minor peaks of 15.4, 14.8 and 15.5 pg/ml on days +1, +2 and 0 at 03.00, 09.00 and 09.00 hr, respectively. Whereas the progesterone levels were maximum on day 3 before estrus and showed decreased gradually (P<0.01) on day one at around ovulation [34].

Influence of age, weaning, season and body weight on the levels of progesterone, estradiol-17β and LH in growing buffalo heifers: The influence of age, weaning, season and body weight on the peripheral levels of progesterone, estradiol, and LH were studied during neonatal, perinatal and peripubertal periods in buffalo heifers. All buffaloes exhibited estrus only after 30 months of age and had higher levels of LH and estadiol and a lower level of progesterone on the day of estrus. The progesterone levels were affected significantly (P<0.01) by different seasons, by weaning (P<0.05) whereas the estrogen levels was affected significantly (P<0.01) by weaning and varied at different seasons and body weights. Great variations in the LH levels was found significantly (P>0.01) between ages and body weights [36].

Hormonal profiles of Follicle Stimulating Hormone, Progesterone, and Estradiol- 17 β in an estrus rural buffaloes

Twelve Murrah type buffaloes between ages 4-7 years were selected for monitoring hormone profiles by RIA technique. The samples were collected at a weekly interval for a period of 28 days. The FSH Levels and estradiol- 17-β fluctuated in between 5.5-6.2 ng/ml to 7.6-15.0 pg/ml, respectively. Whereas the circulatory levels of progesterone ranged from 2.3 ng/ml to 4.3 ng/ml. However, the progesterone levels fall dramatically to 0.4 ng/ml with a sharp increase of FSH (48.5 ng/ml) and estradiol-17β (50.1 pg/mg) on the day of estrus [37].

Postpartum levels of estradiol-17 β in crossbred cows retaining fetal membranes

Circulatory levels of estradiol-17 β were estimated in 15 Cross bred cows of KS, KF and 10 animals without retaining fetal membrane. The hormone was estimated on days 0 considered to be between 6-24 hr of partum, 1, 3, 5, 7, 15, 20, 30, and 40 days of partum. A level of 30.0 pg/ml and 17.1 pg/ml was recorded on the day of parturition in animals without retaining FM. The levels then gradually decreased in both the groups after partum. The study indicated that a lower level of estradiol-17 β is probably responsible for the retention of FM in cross bred animals of KS & KF [38].

Gonadal hormone profiles in super ovulated buffaloes and goats

Progesterone hormone profiles were monitored in Murrah buffaloes super ovulated with follitropin on days 110-12 at 12 hr apart. The concentration varied from 2.23 to 7.74 ng/ml before administration of follitropin and started declining 24 hr after PGF2 alpha administration on day 13 of the cycle showing a minimal level of 0.33 ± 0.16 ng/ml on the day estrus which gradually increased to 6.87 ± _3.46 and 9.06 ± 1.68ng/ml during mid-cycle as in [39]. Similar trials were also done in 1986 in goats super ovulated with PMSG, HCG and PGF2α where a level of estradiol-17 β) was observed from 4.16 to 10.94 pg/ml and a higher concentration of 15.10 to 60.11 pg/ml in goats having a follicles of 2-8 mm diameter during the entire period of trial from day 0 to day 10. However, a very low level of progesterone were shown ranging from 0.11 on day 3 and 0.80 ng/mg on day 10 of the cycle [40].

Prolactin hormone

The prolactin levels were significantly higher on the day of estrus and parturition which subsequently declined during sub estrus and post parturient periods. However, the levels in an estrum heifers were about 3 to 4 fold higher (104.80 ± 17.23 to 165.72 ± 22.56 ng/ml) when compared with the levels during estrous cycle among repeat breeding cycling buffaloes (21.99 ± 0.72.59 to 56.0 ± 33.81 ng/ml [41].

Hormonal profiles during different phases of reproduction in goats

Peripheral concentration of plasma progesterone, estradiol-17β and cortisol were monitored during estrous cycle, early pregnancy, gestation, parturition and postpartum periods in goats. The progesterone levels were very low (0.25 ± 0.15 ng/ml) on the day of estrus which gradually increased to a peak level of 2.77 ± 1.18 ng/ml during the late luteal phase and dropped sharply on day 19 of the estrus cycle in non-pregnant goats. The levels continued to increase up to day 75(6.2 ± 0.61 ng/ml) of gestation in pregnant goats and then a sudden withdrawal on the day of kidding was observed. Contrary to it the levels of estradiol-17 β and cortisol showed different trends and were higher on the estrus with a gradual decrease during early pregnancy followed by a sharp increase on the day of kidding. However, the progesterone in whole milk was 10 times higher than in blood plasma during early pregnancy. The study indicated that the monitoring of hormones by RIA is useful for monitoring the micro levels of hormones responsible for exhibiting reproductive events and early pregnancy diagnosis (EPD) in goats [42].

Plasma Progesterone levels during pregnancy in goats

The progesterone concentrations in the blood plasma of pregnant goats of mixed breeds during post 90 days of gestation fluctuated between 1.9 ± 0.24 and 2.57 ± 0.59 ng/ml during late pregnancy but declined on the day of parturition, with a further slight decline during the postpartum period [43].

Plasma esradiol-17 β levels during pregnancy and peripartum in goats

The plasma estradiol-17 β levels continued to decrease during early pregnancy of day 1to (9.72 ± 5.23 to 5.93 ± 1.78 pg/ml and an average level with a range of (17.84 ± 3.00 to 6.33 ± 3.12 pg/ml) after day 9 to day 17) were observed with a dramatic increase on 43.88 ± 15.86 pg/ml prior to day of kidding which continued to decrease for 19 days postpartum. Therefore it is derived that a sudden increase in estradiol-17 β levels is responsible for the act of parturition in goats [44].

Hormonal profiles in induced parturient goats

The blood samples were drawn on days 23, 20, 17,14,11, 8, 5, 2, 1(before kidding), on the day of kidding(day 0) and also after kidding on days 1, 2, 4, 7, 10, 13, and 16. An increase ($P<0.05$) in the levels of cortisol an estradiol17B and decrease in progesterone in all groups was observed on the day of kidding. However, no increase in cortisol concentration was recorded in goats induced with dexamethazone on the day of kidding. The study suggested that the prepartum pregnancy can be terminated successfully with PGF2α and corticoids in goats as in [9].

Future Plans

The recent techniques and strategies such as use of sexed germ plasm, stem cells, DNA ancestry, automated heat detection devices, sex determination by lacer detection techniques,(LTD), and feeding of somatotropin (bST) hormone are some more which needs to be incorporated in such programmes. However, use of genomics, proteomics and bioinformatics will undoubtedly provide researchers with a greater understanding to enhance efficient reproductive process in animals. The other emerging assisted parameters will be the incorporation of zoo noses and public health aspect.

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