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A REVIEW OF CURRENT BOVINE OBSTETRICAL PRACTICES

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ABSTRACT

The dairy animal population especially buffalo suffers from various obstetrical problems, the solution for some of these have been devised and are in process for the others. There is need to understand management factors associated with uterine torsion in buffaloes, the physiological and cardiovascular impact of dystocia. This will help in timely resuscitation of dystocia-affected dam through the use of tranquilizers, glucocorticoids plus anti-oxidants and crystalloid plus colloid based fluid therapy. Huge success has been achieved in cervical massage or intracervical treatment for dilatation of incompletely dilated cervix as well as timely and appropriate handling of an obstetrical case by understanding the importance of time factor. The use of uterine lubricants improves success rate. Fetotomy should be used as preferred obstetrical procedure by making the availability of an appropriate Fetotomy kit and the knowledge of 'Strength-Stamina-Skill' for carrying out fetotomy procedures.

Keywords: Buffalo, Doppler, Fetotomy, Obstetrics, Uterine torsion

For a veterinarian and a dairy farmer, getting a cattle or buffalo pregnant is very tough job, followed by health care during pregnancy. Unfortunately, if an animal suffers from difficult calving at the end of gestation period and the case is not handled properly, then the outcome may be death of fetus or dam or both. This leads to financial and emotional burden on the farmer. In case of survival of dam, if an obstetrical case is handled carefully there will be meagre gynaecological complications later on. In a study, available 34% conception data revealed that 90% buffaloes treated within 36h of occurrence of uterine torsion conceived successfully (Singh *et al.,* 2017). Considering the importance of bovine obstetric care, the present paper has focused on effective strategies that can be adopted for the judicious delivery of a calf and save the life of dam.

Management factors associated with uterine torsion in buffaloes

Uterine torsion is the major cause of dystocia in buffaloes which warrants immediate attention. Various existing suppositions concerning the maternal and the fetal destabilizing factors liable for the occurrence of uterine torsion in buffalo are unrealistic, however some of these have been justified by logical interpretations. Nevertheless, buffalo reared in open housing system and those reared by nomads in open grazing system rarely encounter uterine torsion. In addition, Indian Murrah buffalo imported by Brazil in 1960s and reared in big pastures on hills, never suffer from uterine torsion. In fact, a study involving 570 buffalo farmers revealed that extensively reared buffaloes were at lower risk of uterine torsion as compared to the stall-fed buffaloes (Singh *et al.,* 2017). Daily exercise in the form of walk/ wallowing reduced the risk of uterine torsion. Keeping buffaloes on kacha floor is associated with lower risk of uterine torsion. However, segregation of advance pregnant buffaloes and feeding practices had no impact on incidence of uterine torsion (Singh *et al.,* 2017). This suggests the possibility of poor musculature, due to failure of exercise, in buffalo suffering from uterine torsion as these buffalo usually belong to farmers who rear buffalo in closed/tie housing system. Thus, buffalo farming community can be advised to expose buffalo to free movement for some period of the day so that perineal/abdominal muscles are strong and well

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developed.

Understanding the cardiovascular impact of dystocia

Doppler ultrasonography-aided assessment of uterine blood flow in relation to duration and degree of uterine torsion was carried out in cattle (Singh *et al.,* 2016a). Fourteen dairy cattle with uterine torsion were detorted and fetal delivery was completed within 30 min after detorsion. Six live calves were delivered by cattle having torsion from lesser duration and rest dead calves delivered by dams with prolonged uterine torsion. Whereas the dams of majority of live (n=4/6) or dead (n=5/8) fetus had uterine torsion ≤180° or >180°, respectively. Doppler ultrasonography of middle uterine artery ipsilateral (IpsiUA) and contralateral (ContUA) to the side of torsion was carried out before uterine detorsion for doppler indices viz. blood flow volume (BFV), time-average peak velocity (TAP), resistive index (RI) and pulsatility index (PI). With increase in degree and duration of torsion, BFV in both IpsiUA and ContUA was reduced. In long standing uterine torsion, TAP values were found lower as compared to short duration torsions in both ipsilateral and contralateral uterine arteries. In ipsilateral uterine artery PI (PI-IpsiUA) increased with an increase in duration of torsion. The presence of Pre-diastolic notch in IpsiUA and ContraUA validates the hindrance in blood flow through the vessel and absence of diastole in higher degree and/or duration uterine torsion defined the severity of torsion which further relates to fetal viability (Singh *et al.,* 2016a). This suggested that assessing the blood flow parameters in middle uterine artery in relation to degree and duration of uterine torsion can serve as useful prognostic indicator. The cattle having lesser degree of uterine torsion could have more chances of fetal survival due to lesser alterations in blood flow.

Furthermore, the uterine blood flow during peripartum period in buffalo was associated with shedding of placenta (Singh *et al.,* 2016b). The values of TAP and BFV in the uterine artery recorded at the start of calving process invariably exhibited a decreasing trend by 30 min post-calving which continued till 6 h post-calving. The values of RI and PI in uterine artery ipsilateral to side of pregnancy increased consistently till 6 h post-calving as compared to pre-calving values. At 6 h post-calving, both in IpsiUA as well as ContUA, the values of TAP and BFV were higher, and RI and PI were lower in buffalo that failed to shed placenta within 6 h post-calving in comparison to their counterparts taking >6 h after calving for shedding placenta (Singh *et al.,* 2016b). Thus, the reference values for doppler indices for uterine artery during peripartal period were generated and it was observed that the decrease observed in blood flow in uterine artery was slow in buffalo with delayed shedding of placenta.

Understanding the physiological impact of dystocia

The occurrence of dystocia and the extent of obstetrical manipulations are highly stressful and are directly reflected through an increase in adrenal activity. In dystocia-affected buffalo, plasma cortisol is much higher compared to their normal calving counterparts (41.61±4.18 *vs.* 8.65±1.14 ng/ml; Ghuman *et al.,* 1998). Furthermore, applying uterine detorsion procedures exhibits clear impact on plasma cortisol with excessive adrenal stimulation due to one to two extra detorsion rolls (Ghuman *et al.,* 1997b). The survival rate of buffalo requiring 1-2 or 3-4 rolls for complete detorsion of uterus was 85 and 43%, respectively (Ghuman *et al.,* 1997b). The type of obstetrical procedures also impacts the physiology of the dam. The release of plasma cortisol in buffalo subjected to caesarean was more and for longer duration than their counterparts subjected to fetotomy, thus, leading to poor survival rate in buffaloes following delivery of calf through caesarean section (25-95%) compared to per-vaginal delivery (88-100%; Ghuman and Dhaliwal, 2011).

Resuscitation of dystocia-affected dam

Tranquilizers: Tranquilization of buffalo before detorsion of uterus failed to prevent the stressful impact of uterine torsion (Ghuman *et al.,* 1997a). Moreover,

during post-detorsion period, alleviation of stress was poor in buffalo detorted under chlorpromazine and diazepam tranquilization. In fact, tranquilization with chlorpromazine and diazepam had paralyzing action on skeletal muscles leading to recumbency for longer duration (Ghuman *et al.,* 1997a). This warrants more studies on tranquilization therapy for alleviating the stress of dystocia.

Glucocorticoids plus anti-oxidants: Dystocia affected buffalo administered dexamethasone sodium (40 mg, *i.v.*) before relieving dystocia and subsequently once daily for 2 days postpartum lead to significant reduction in plasma cortisol on Day 1 postpartum, thus suggesting that Hypothalamus-Pituitary-Adrenal axis was responsive to dexamethasone-induced negative feedback during the immediate postpartum period following dystocia (Sathya *et al.,* 2005). A decline in plasma cortisol, erythrocytic malondialdehyde level and superoxide dismutase activity was greater in vitamin E and Selenium supplemented dystocia-affected buffalo (78.62±2.7%, 13.38±9.75% and 28.83±7.72%, respectively) compared to their unsupplemented counterparts (54.33±12.62%, 3.48±4.16% and 17.69±12.93%, respectively; Sathya *et al.,* 2007), thus suggesting that providing antioxidant supplementation is beneficial in reducing oxidative stress in dystocia affected buffalo.

Crystalloid plus colloid based fluid therapy: The delay in handling of dystocia leads to progression of dam towards dehydration and toxaemia due to decrease in plasma and blood volume. Intravenous fluid administration is considered best method for treating dehydrated bovines. In routine dystocia cases, a meager amount of intravenous normal saline therapy (NSS) is used. However, a large amount of NSS is required as the bovines suffering from mild to severe dehydration (8-12%) are recommended around 50-120 ml NSS/kg b wt, which is a huge amount and not practicable to administer due to exuberant cost, catheterization of vein, prolonged restraint and periodic monitoring. The possible way out is the use of large volume of oral fluid (fresh water) with small volumes of intravenous hypertonic saline either with or without Dextran-40 (7.2% HSS @ 4ml/kg + Dextran-40 @10ml/kg + Oral fluid 25L). Intravenous administration of Dextran-40 is necessary to maintain elevated plasma and blood volume for longer durations and thus decrease the degree of dehydration. Thus, it was recommended that administration of fresh water through oral route along with intravenous hypertonic saline and Dextran-40 could be a quicker, practical, easy and effective method for resuscitating the dystocia affected buffaloes suffering from variable degree of toxaemia and hypovolemia, and thereby decreasing their mortality rate (Kumar *et al*., 2009a, b).

Cervical massage or intracervical treatment: The challenge of achieving complete cervical dilatation in successfully detorted uterine torsion affected buffalo carrying dead fetus can be taken care by cervical massage with SCMC, otherwise leaving the soft or moderately soft cervix on its own to dilate will lead to hardening of cervical texture followed by its failure to dilate. In a study, a procedure of manual dilatation of cervix was developed for buffalo in which cervical massage for 15 minutes (3 times at hourly interval) can be carried out using warm SCMC (Honparkhe *et al.,* 2009). Using this procedure, cervix can be dilated in all the buffalo with soft cervical texture, whereas success rate up to 50% can be achieved in buffalo with moderately soft cervix. Out of buffalo with soft cervical texture and not being subjected to cervical massage, only 29% achieved cervical dilatation whereas none of the buffalo with moderately soft cervix achieved cervical dilatation. In the absence of cervical massage, soft cervical texture was converted to hard texture within 24 h following detorsion of uterus and subsequently cervix failed to dilate (Honparkhe *et al.,* 2009). In another study, 24 buffaloes with incomplete dilation after successful detorsion were subjected to different cervical dilation treatments. The complete dilation of cervix occurred in buffaloes (87.5%) treated intracervically with hyaluronidase enzyme, whereas Prostaglandin E1 led to dilation in 62.5% of buffaloes

(Singh *et al.,* 2017).

Timely and appropriate handling of an obstetrical case

Importance of time factor: An obstetrical case handling in the field by quacks is a major constraint behind the poor survivability of bovines. Depending upon injudicious handing, survival rates of torsion affected bovines presented in <36 h, 36-72 h and >72 h of occurrence of torsion are 52-86, 29-74 and 32-62%, respectively (Ghuman, 2010). Cases around lower range of the survival rate are those that are first handled in the field. In fact, survival rate in torsion affected bovine declines linearly (from 87 to 43%) with an increase in duration of uterine torsion (Ghuman, 2010). The duration of uterine torsion and the time taken for complete dilatation of cervix increases the severity of uterine necrosis, fetal putrefaction, maternal toxemia, dehydration, shock and peritonitis. The buffalo that ultimately died following detorsion and the buffalo that delayed the fetal delivery following detorsion had prolonged elevated plasma cortisol as compared to surviving counterparts and the early delivering counterparts, respectively (Ghuman *et al.,* 1998). This warrants creating awareness among farmers and field practitioners' for timely and appropriate handling of an obstetrical case.

Uterine lubricant: The use of adequate amount of uterine lubricant allows the correction of fetal malpresentation and helps in carrying out procedures like fetotomy for successful per-vaginal delivery. In fact, for appropriate handling of dystocia, the importance of uterine lubricant can be understood from the fact that by the time, an obstetrical case is presented in a referral hospital, the birth passage becomes dry due to continuous contraction of the uterus and the previous handling of case through repeated vaginal examination. Moreover, the uterus gets contracted on the fetus following the expulsion of uterine fluid. In the absence of uterine lubrication, fetal delivery through fetal mutations by an obstetrician becomes impossible. Thus, if the birth passage is dry, then copious volume of a non-irritant lubricant like sodium carboxy methylcellulose (SCMC, a commonly available chemical from laboratory chemical suppliers) is required for birth passage lubrication. One per cent solution of SCMC can be prepared by boiling 200 ml of clean water and slowly adding 10 gram SCMC powder while stirring (Ghuman, 2015). Additional clean water can be added while stirring to bring the total volume to a litre. Few crystals of potassium permanganate can be added to the gel solution. This product is extremely slippery, and good footing is essential. A sterile stomach tube and pump are used to gently instill the mixture into the uterine lumen.

Fetotomy as preferred obstetrical procedure

Fetotomy is usually advantageous because dam survival rate is high following fetotomy compared to caesarean, future fertility is not compromised, post-operative complications are less and excessive stress to dam associated with forced traction is avoided (Singh *et al.,* 2013). Fetotomy is usually indicated in case of emphysemated fetus to avoid the risk of peritonitis following caesarean operation as well as in cases of fetal malpresentation that can not be corrected by mutation (Hip-lock, Breech presentation), feto-pelvic disproportion (narrow pelvis, oversized fetus) and in case of partially dilated cervix (Ghuman, 2010). However, it should be considered that fetotomy is a time-consuming process, exhaustive for the obstetrician and there is risk of injury to obstetrician and dam. Moreover, fetotomy should be avoided in case of tear in the birth canal of dam as the tear can be aggravated with fetotomy instruments and may prove fatal for dam.

Fetotomy kit: The non-availability of good quality fetotomy kit is a major constraint and hence some obstetrical cases, which can be relieved through per-vaginal delivery, are subjected to caesarean operation. In the kit, a good quality Thygesen's fetotome is a prime necessity. A poor quality fetotome usually leads to breakage of fetotomy wire due to failure of fetotome to allow free movement of wire. The second major kit

component is a strong and flexible fetotomy wire (27 interwoven steel wires in a group of 3 x 3 x 3 wires) which is required to compete the cut through soft and bony tissues of the fetus (Ghuman, 2014). The non-availability of this fetotomy wire in local market is also a constraint, as the wire needs to be imported. Other important fetotomy kit components are calving rope carrier, guarded knife and eye hooks.

Strength-Stamina-Skill: The perfection in fetotomy depends on technical knowledge, adequate training and experience, correctly designed instruments and proper lubrication. Clinician intending to carry out fetotomy should consider the rule of 3S viz, 'Strength-Stamina-Skill' for completing a fetotomy procedure and the technical knowledge regarding where the cut(s) should be made. If clinician is not familiar with correct technique then the best option is caesarean section (Ghuman, 2010). A common fault is to choose fetotomy only after the birth canal has already been traumatized by unproductive attempts at manual correction. Avoid manipulating dry birth canal as this may lead to uterine rupture and ultimately unsuccessful fetotomy. The obstetrician should ensure that the wire threaded around the fetal part to be amputatd is not crossed or kinked, head of the fetotome is in the correct position, cover fetotome head with a hand and avoid producing sharp skeletal edges as they may damage the birth canal. An assistant is instructed to start the cut by slow, short, to-and-fro arm movements. Longer movements decrease the amount of heat generated and spread the wear on the wire. Minimize the number of cuts required for delivery of all the fetal parts. This will shorten the intervention time and permit a less traumatic delivery of dead fetus.

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PURIFICATION OF SPERMATOGONIAL STEM CELLS FROM RAM TESTICULAR ISOLATE USING FICOLL DENSITY GRADIENT SEPARATION

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ABSTRACT

Spermatogonial stem cells (SSCs) can be used to propagate male superior germ plasm and to preserve the male of high genetic merit and endangered species. The objective was to assess the efficiency of different Ficoll density gradient separation in order to purify SSCs from the testicular cell isolates. The mixed testicular isolates obtained after enzymatic digestion of testicular tissues was subjected to two different Ficoll density gradient methods (method 1: Ficoll at 10 and 12% and method 2: Ficoll at 10, 12 and 16%). The percentage of SSCs (positive for PLZF, an SSC marker) obtained from the fractions, F12 media and F12-16 Interphase (FI) yielded ($p<0.05$) higher percentage of PLZF+ spermatogonia as compared to initial testicular isolate (35.1±3.8 and 22.8±4.5 *vs* 11.2±3.7). The viability (%) of F12 and FI enriched fraction was 55.6±4.3 and 51.2±6.5, respectively. In brief, Ficoll purification method using F12 fraction yielded higher ($p<0.05$) recovery rate ($4.9±1.2x10^6$ cells/g of testis) with improved purity ($1.8±0.4x10^6$ PLZF+ cells) when compared to FI (recovery rate: $3.28±1.2x10^6$ cells/g of testis and purity: $0.8±0.3x10^6$ PLZF+ cells) and can be used either alone or in combination with other purification methods for further enrichment of SSCs that can be used for culture.

Keywords: Ficoll density gradient, PLZF positive, Ram, Spermatogonial stem cell, Testis

INTRODUCTION

Spermatogonial stem cells (SSCs) possess a vital role in male reproduction, genetics and transgenesis studies, however, the successful purification of SSCs from testicular isolate is essential for culture and transplantation (Binsila *et al*., 2017). The testis consists of different types of cells like Sertoli cells, Leydig cells, SSCs and different stages of differentiating germ cells with the percentage of SSCs to be <1% of testicular cells. Attempts were made to isolate and purify SSCs with high viability. Although, the isolation and culture of SSCs has started in bovine, caprine, porcine and bubaline (reviewed in Zheng *et al.,* 2014), the isolation, culture and transplantation of SSCs in livestock is in a nascent stage. Considering the less population of SSCs in the testicular cell isolate, several enrichment procedures were tried to obtain a maximum population of SSCs with appropriate quality which is a prerequisite for downstream applications such as culture experiments and or transplantation. The enrichment procedures include magnetic-activated cell sorting (MACS), fluorescence activated cell sorting (FACS), differential plating, selection with extracellular matrix (ECM), velocity sedimentation and density gradient centrifugation (Borjigin *et al*., 2010; Valli *et al*., 2014). In addition, the combination of enrichment techniques may augment the purity of spermatogonia (Herrid *et al*., 2009). As the initial isolate of testicular cells contains cells of varying size and density, the density gradient separation can be effectively used to enrich SSCs. Recently, the Ficoll density gradient technique provided optimum purity with high viability (Joseph *et al*., 2017). Hence, the objective of present study was to isolate and enrich SSCs from the ram testes using Ficoll density centrifugation.

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MATERIALS AND METHODS

Isolation of putative SSCs: Prepubrtal ram testes samples (n=6) procured from the slaughterhouse were transported within 2 h in ice-cold saline to the laboratory. After removing tunica vaginalis and epididymis, testes tissue samples were excised mechanically, cut into small pieces and testicular cells were isolated immediately using enzymatic methods (Izadyar *et al*., 2002; Borjigin *et al*., 2010) with slight modifications. In brief, testes tissue (2 g) was washed using Dulbecco's Modified Eagles Medium (DMEM) and placed in DMEM. The tissues were mechanically sliced into small pieces, ground with syringe plunger to form homogenized tissue mass. The homogenized samples were added with 4 ml DMEM containing collagenase (2 mg/ml) and incubated for 1 h at 37°C. The digested tissue was washed twice with Dulbecco's phosphate buffered saline (DPBS) by centrifugation at 269 g for 5 min and the supernatant was removed. The pellet containing digested tissues was added with trypsin (0.5 mg/ml) in 5 ml DPBS and incubated at 37˚C for 5-7 min followed by inactivation of trypsin with the addition of an equal volume of DMEM containing 5% fetal bovine serum (FBS). The digested tissues were filtered through 70 µm strainer (352350, BD Falcon) and the filtrate containing cells were washed using DMEM twice by centrifugation at 269 g for 5 min and the supernatant was discarded. The cell pellet containing mixed testicular cells was suspended in DMEM containing 5% fetal bovine serum (Initial cells isolate, IC).

Enrichment using Ficoll density gradient separation: Putative SSCs from the IC were enriched using Ficoll density gradient centrifugation (Panta *et al*., 2011) with modifications. Briefly, two different gradients were carried out viz. Ficoll at 10 and 12% and Ficoll at 10, 12 and 16%. The isolated cells from each gram testes were mixed in 2 ml of 10% Ficoll in DMEM and slowly layered on to the top of 2 ml of 12% Ficoll in DMEM in a 15 ml centrifuge tube. The tubes were centrifuged (5810R, Eppendorf, Germany) at 800 g for 30 min at 18˚C. From 10 and 12% gradient (method 1), 12% Ficoll fraction (F12: pellet and 12% media) and from 10, 12 and 16% gradients (method 2), the interphase of 12 and 16% fraction (FI) were collected. These fractions were enriched in SSCs based on our preliminary study. F12 and FI fractions were centrifuged at 1680 g for 5 min. Supernatants were discarded and the pellet was washed in the media (DMEM added with 5% FBS) by centrifugation at 269 g for 5 min. The supernatant was removed and pellet was re-suspended in the media. Ficoll enriched cells were analyzed for the stem cell property using marker Promyelocytic leukemia zinc finger protein (PLZF), viability and concentration (Figure 1).

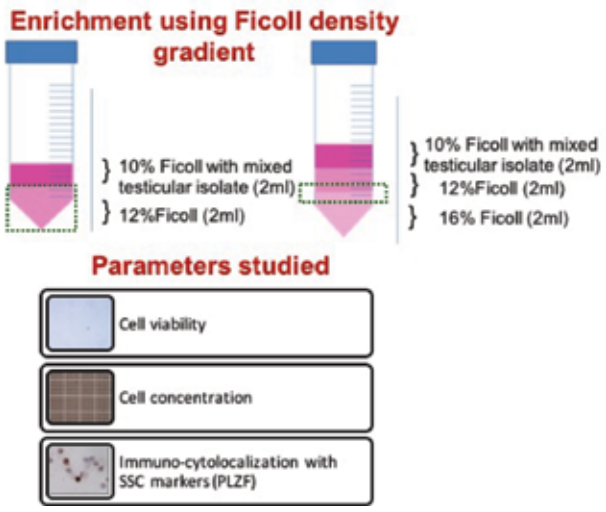

Figure 1: Steps followed for the enrichment of ram SSCs using Ficoll density gradient separation. The initial testicular isolate was subjected to Ficoll density gradient (10 and 12%; 10, 12 and 16%) separation. The SSCs were enriched in 12% Ficoll fraction (F12: Pellet and 12% media; FI: interphase of 12 and 16%).

Localization of different SSC markers: The purity of putative SSCs in different fractions of Ficoll separation was assessed using PLZF (SSC marker) localization in the initial isolate and enriched fractions (Somashekar *et al*., 2017) with slight modifications. Briefly, the smears were fixed with 4% paraformaldehyde for 10 min and rinsed with TBST (Tris-buffered saline with

0.05% Tween 20). Then the slides were incubated in 0.01% Triton-X in TBS for 5 min and washed with TBS. The slides were incubated in TBS containing 0.6% H_2O_2 for 20 min. The nonantigenic sites were blocked by adding a solution of 3% BSA in TBST onto the cells and incubating at room temperature for 30 min. The smear was added with primary antibody (rabbit polyclonal IgG: PLZF, 1:100 dilution, sc-22839, Santa Cruz Biotechnology, Santa Cruz, USA) and incubated overnight at 4°C. After incubation, the slides were washed three times using TBST for 5 min each and incubated with secondary antibody (105499, Goat anti-rabbit IgG-HRP, GeNei, India) for 30 min at room temperature. Then the slides were washed three times with TBST for 5 min each and incubated with DAB substrate for 10 min for the development of brown color. For negative controls, the smears were incubated in the buffer (TBST) without primary antibody. The cells were counterstained with hematoxylin. The cells were observed under 400X magnification using a phase contrast microscope (Nikon 80i, Nikon, Japan) and the percentage of cells positive for PLZF activity was calculated.

Viability of isolated and enriched cells: The viability of the isolated and enriched cells was assessed by trypan blue (0.5%) staining. Equal volume (5 µl) of trypan blue and cells suspension in medium were mixed and incubated for 2 min and observed at 400X magnification in a phase contrast microscope (Nikon 80i, Nikon, Japan). The cells with blue color were considered as dead and those did not take up the stain were considered as viable and the percentages of viability were calculated.

Cell concentration: The number of cells obtained following isolation and enrichments was calculated using hemocytometer and the total number of cells /g of testis were estimated. Based on the immunocytochemistry (ICC) results, the number of SSCs yield /g of testis was calculated in the initial isolate and enriched fraction.

Cell morphology documentation: The cell morphology was estimated in the initial isolate and enriched fractions using a phase contrast microscope (Nikon 80i, Nikon, Japan).

RESULTS AND DISCUSSION

The present study was carried out to enrich ram SSCs from initial testicular isolate using Ficoll density gradient separation. In the method 1, SSCs (PLZF+ cells) were enriched in 12% Ficoll fraction (F12: pellet and 12% media; 35.1±3.8%) and using the method 2, Ficoll at 10, 12 and 16% gradients, the SSCs were enriched in the interphase of 12 and 16% fraction (FInterphase; 22.8±4.5%; Table1). The percentage of PLZF+ cells in F12 fraction was higher ($p<0.05$) than the initial isolate, however, did not differ ($p>0.05$) with FI fractions. The morphologically enriched cells were round in shape with a homogenous size (Figure 2a). In the present study, 3.6 (FI) and 4.1 (F12) Fold enrichment obtained through Ficoll gradient separation

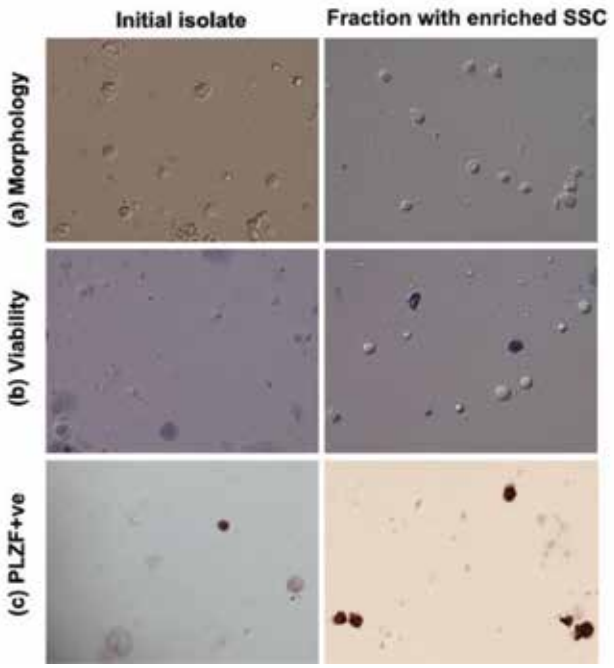

Figure 2: (a) Morphological appearance of pre-enriched and enriched (round in shape with a homogenous size) SSC fractions, (b) The cells stained with trypan blue are dead and others are live, and (c) The putative SSCs (brown colour) were identified using PLZF marker. 400x

Table 1: Enrichment of putative spermatogonial stem cells (SSCs) from the initial ram testes isolates (n=6) using Ficoll density gradient centrifugation. Method 1: 12% Ficoll fraction (F12: pellet and 12% media), Method 2: Interphase of 12% and 16% fraction (FI). PLZF, Promyelocytic leukemia zinc finger protein.

Parameters	Initial isolate	Ficoll enrichment	
		Method 1	Method 2
Total no. of cells (x10^6) /g of testis	17.7±2.8	4.9±1.2	3.3±1.2
Viability, %	72.0±4.1^{c}	51.2±6.5^{ab}	55.6±4.3^{a}
PLZF^{+ve}, %	11.2±3.7^{a}	35.1±3.8^{bc}	22.8±4.5^{ab}

^{a,b,c}p<0.05

is in agreement with the 2.9 fold in bovine (Herrid *et al*., 2009) and 3.6 fold in sheep (Rodriguez-Sosa *et al*., 2006) through percoll enrichment. Further, the inclusion of density gradient separation along with differential plating method improved the purity of SSCs (42.70 to 64.39% GFRα+ cells) in cats (Tiptanavattana *et al*., 2015).

The viability of purified fraction following Ficoll density gradient though reduced (p<0.05) as compared to initial isolate, did not adversely affect the culture procedure (unpublished data; Table 1, Figure 2b). Similarly, an earlier study reported reduced viability of enriched bovine SSCs obtained through Percoll density gradient centrifugation (de Barros *et al*., 2012). The average yield of number of mixed testicular cells from each gram of testicular tissue was 17.67x10^6 and

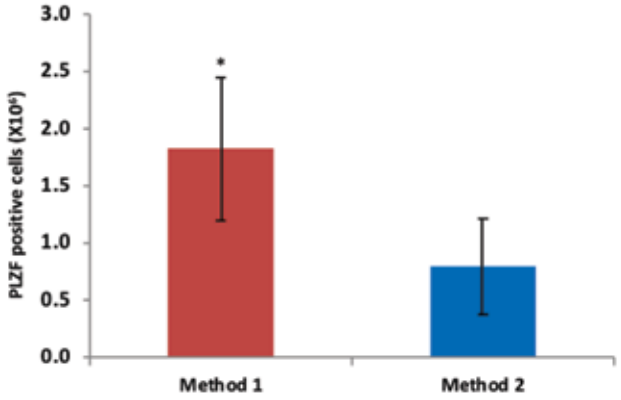

Figure 3: Total number of PLZF^{+} cells (x10^6) in 12% Ficoll fraction (F12: pellet and 12% media, Method 1) and in the interphase of 12% and 16% fraction (FI, Method 2) of ram testes isolates (n=6). *p<0.05.

the purified fractions, F12 and FI yielded 4.85x10^6 and 3.28x10^6 cells from prepubertal ram, respectively (Table 1). The total number of PLZF+ cells in F12 and FI fractions were 1.8±0.4 and 0.8±0.3 million, respectively (Figure 3).

The study revealed that even though there was no significant difference in PLZF+ cells between the two enriched fractions (F12 and FI), the F12 fraction was better based on higher recovery rate. Hence, F12 fraction of 10 and 12% Ficoll density gradient may be used for the culture of SSCs. Through PLZF localization study, it was revealed that SSCs can be enriched by Ficoll density gradient separation and the procedure is suitable for eliminating the other testicular cells like differentiating spermatogonial population and Sertoli cells as these cells will be separated out in different layers based on the density (Figure 2c). A similar study for the enrichment of SSCs using Ficoll density gradient was reported in rodent (Jeong *et al*., 2003) and fish (Panda *et al*., 2011). Percoll density gradient though chemically different, but separates cells based on density and has been used for SSCs isolation in different studies (Izadyar *et al*., 2002; Tiptanavattana *et al.,* 2015). As the initial isolate of the testicular cells contains cells of varying size and density, density gradient separation might be a suitable option to segregate the subsets of homogenous cells. The present study, though provide the optimum number of PLZF positive cells for culture, the SSCs enriched fraction from density gradient separation can be combined with other protocols like differential plating (Izadyar *et al*., 2002; Tiptanavattana *et al*., 2015) or

magnetic activated cell sorting (MACS) (Panda *et al*., 2011) for improving enrichment efficiency. Another concern from the experiment is the reduced viability of isolated cells. The viability of the cells may be probably improved by reducing the duration of experimental procedures.

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RELATIONSHIP BETWEEN SERUM PROGESTERONE AND ESTRADIOL-17$_\beta$ AND EMBRYONIC LOSS IN FRISIAN COWS UNDER EGYPTIAN CONDITION

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ABSTRACT

Association between circulating progesterone and oestradiol-17$_\beta$ concentrations and embryonic loss in Frisian cows (n=105) under Egyptian condition was investigated. Total embryonic losses in present study were 26.4%. On d25, progesterone (P_4) concentrations and sonography revealed 79.3% cows were continuing gestation and 20.7% lost their pregnancies (early embryonic loss, EEL). Furthermore, 7.4% cows with late embryonic loss (LEL) were detected between d25-42. An increase in P_4 levels started from day 4 to 7 in cows diagnosed pregnant compared to low P_4 in cows that lost pregnancy ($p<0.05$). Cows with LEL had decrease in P_4 from d28. Estradiol (E_2) concentrations increased ($p<0.05$) in cows with EEL starting from d4 to 21 after AI compared to cows diagnosed pregnant. Starting from d4 to 40 after AI, P_4/E_2 ratio was higher ($p<0.05$) in cows diagnosed pregnant compared to cows exhibiting EEL loss, while cows with LEL had highest P_4/E_2 ratio till d25 post-AI and declined from d28 to 40.

Keywords: Embryonic loss, Friesian cows, Estradiol-17$_\beta$, Pregnancy, Progesterone

INTRODUCTION

There is no practical way to reduce the early pregnancy loss in dairy cattle, however the management strategies may be helpful in increasing the calving rate. Prior to the development of ultasonography for pregnancy diagnosis, it was difficult to determine the viability of fetuses at an early stage of gestation (Chaudhary and Purohit, 2012). Moreover, an estimation of early pregnancy loss by progesterone (milk or blood) can not be detected with certainty. The aim of present study was to assess the embryonic loss with the help of transrectal ultrasonography and serum progesterone (P_4) and estradiol (E_2) determination in Frisian cows under Egyptian condition.

MATERALS AND METHODS

Friesian cows (n=105; age, 30-96 mo.; b. wt., 440-660 kg) were used in this study which was carried out between Oct, 2012 to July, 2013. The tagged experimental animals were fed on concentrate feed mixture, maize silage and rice straw in summer. While, in winter, animals were fed concentrate feed mixture, fresh berseem and rice straw according to their body weight and milk production. All heifers were free from any disease with healthy appearance and were housed in separated two groups under semi-open sheds, partially roofed by asbestos.

Estrus was visually detected two times daily between 6 a.m. and 7 p.m. using a teaser bull. Cattle detected in estrus in morning were recorded and artificially inseminated (AI) in the afternoon of same day and cattle detected in estrus in the afternoon were inseminated next morning. Pregnancy was diagnosed by serum progesterone on d4, 7, 11, 13 and 22 post AI, as well as using ultrasonography starting at d22 and d45 post AI.

Blood samples were collected for the determination of progesterone (P_4) and Estradiol-17$_\beta$ (E_2) in blood serum. Within an hour after collection, samples were centrifuged for 15 min at 3000 rpm for serum separation. Serum samples were stored at -20 ºC till the hormonal assay.

A direct radioimmunoassay technique was performed for determination of serum P_4 using

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ready antibody coated tubes kit (Diagnosis Systems Laboratories, Texas, USA). The standard curve of P_4 ranged from 0.0 to 40.0 ng/ml. The sensitivity value was reported as 0.12 ng/ml. The intra and inter-assay coefficients of variation were 8 and 13.1%. Serum E_2 was determined by radioimmunoassay procedure (Sirois and Fortune, 1990). Intra-assay coefficient of variation for E_2 was 4%. Inter-assay coefficient of variation was 10.9%. The detection limit of assay was 2.9 pmol/l.

For diagnosing pregnancy and embryo loss of mated animals, ultrasonographic examinations were performed using a B-mode ultrasound scanner (Falco, Easote/Piemedical, Maastricht, 6-8 MH2 Linear array transducers, Alliance Medical Int.) on day 25 and 42 post AI. The animals were considered pregnant on the basis of presence of anechoic fluid with visualization of embryo and heart beat in either of uterine horns. Early pregnancy loss was confirmed by non-visibility of embryo/fetal heart beat or non-visibility of embryo or the absence of positive signs of pregnancy in an animal previously diagnosed pregnant or the presence of signs of embryo/ fetal degeneration.

The results were statistically analyzed using completely randomized design for the data of diagnostic and therapeutic studies. The significant differences among treatment groups were tested using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

From 105 Friesian cows, 70.8% cows were pregnant on day 25 by both serum P_4 and sonography. Out of these, 79.3% continued their gestation and 20.7% lost their pregnancies by d25 (Table 1). Late embryonic loss was detected in 7.4% from d25-42, thus the total embryonic losses were 26.4% (Table 1). This estimate of early embryonic loss was in close agreement with an earlier study exhibiting 22.8% (Chaudhary and Purohit, 2012).

An increase in serum P_4 started from d4 to d7 in cows diagnosed pregnant ($p<0.05$) compared to low P_4 in cows that lost pregnancy (Table 2). In addition, serum P_4 was higher ($p<0.05$) from d4 to d40 post-AI in pregnant cows compared to cows that lost their embryos. A reduced capacity P_4 secretion can explain around 5% embryonic mortalities in buffalo synchronized and mated by AI during a period of low reproductive activity (Campanile *et al*., 2005). Cows with late embryonic loss exhibited increasing serum P_4 till d25, thereafter, P_4 started to decrease from d28 onward (Table 2). A significant negative correlation was reported between serum P_4 and late embryonic loss as P_4 is responsible for maintaing the pregnancy (Gabor *et al*., 2008).

Serum E_2 increased ($p<0.05$) in cows with early embryonic loss starting from d4 to 21 post-AI compared to cows diagnosed pregnant (Table 3). Estrogen secretion from a large follicle from d14 to 17 of pregnancy may negatively affect embryo survival (Inskeep, 2004). In addition, cows pregnant at day 28 had lower E_2 ($p<0.05$) compared to cows with late embryonic loss (Table 2).

The present results showed that P_4/E_2 ratio was

Table 1: Early (EEL) and late (LEL) embryonic losses in Friesian cows

Parameter	Total	Pregnant		Pregnancy loss	
	N	N	%	N	%
Conception rate, %	105	87	82.8	-	-
EEL, till d 25	87	69	79.3	18	20.7[a]
LEL, d 25-42	69	64	92.7	5	7.4[b]
Total	87	64	73.6	23	26.4

[a,b]$p<0.05$

Table 2: Serum progesterone (P_4) and Estradiol-17$_\beta$ (E_2) concentrations (ng/ml) during post-mating period in Friesian cows. EEL, Early embryonic loss; LEL, Late embryonic loss

Day	Pregnant		EEL		LEL	
	P_4	E_2	P_4	E_2	P_4	E_2
0	0.43±0.05^b	16.36±1.0	0.60±0.04^a	15.79±0.69	0.49±0.06^{ab}	15.65±1.02
4	8.03±0.75^a	4.28±1.57^b	4.40±1.17^b	7.67±1.10^a	7.57±0.65^a	3.66±1.62^b
7	8.98±0.99^a	3.68±0.68^b	4.91±1.17^b	8.23±1.52^a	8.37±0.87^a	3.57±0.84^b
11	9.23±1.23^a	3.17±0.58^b	4.56±1.53^b	11.12±1.81^a	9.06±1.11^a	2.87±1.06^b
12	10.11±0.79^a	3.16±0.70^b	4.61±1.33^b	11.36±1.96^a	9.27±0.86^a	2.96±0.95^b
13	11.20±0.87^a	3.15±0.53^b	6.85±1.55^b	8.67±1.35^a	9.96±0.92^a	3.06±1.27^b
21	9.61±0.76^a	2.81±0.47^b	3.77±0.80b	13.17±2.80^a	8.57±0.78^a	4.56±1.35^b
28	9.09±0.61^a	2.83±0.54^b	-	-	5.15±1.12^b	8.14±1.21^a
40	9.56±0.84^a	2.14±0.43^b	-	-	2.95±0.46^b	5.91±0.96^a

^{a,b}p<0.05, within a column

Table 3: P_4/E_2 ratio during post-mating period in Friesian cows. EEL, Early embryonic loss; LEL, Late embryonic loss

Day	Pregnant	EEL	LEL
0	0.03±0.02	0.04±0.003	0.03±0.03
4	1.87±0.15^a	0.57±0.23^b	2.07±0.20^a
7	2.44±0.32^a	0.59±0.22^b	2.34±0.51^a
11	2.92±1.35^a	0.41±0.24^b	3.17±0.52^a
12	3.19±1.21^a	0.51±0.34^b	3.12±0.45^a
13	3.55±0.98^a	0.79±0.28^b	3.26±0.36^a
21	3.42±0.57^a	0.28±0.11^b	1.88±0.28^a
28	3.13±0.57^a	-	0.63±0.54^b
40	4.46±2.02^a	-	0.49±0.31^b

^{a,b}p<0.05, within a column

higher (p<0.05) in cows diagnosed pregnant starting from d4 to 40 post-AI compared to cows that lost their embryos between d4 to 25 post-AI (Table 3). The cows with late embryonic loss had highest P_4/E_2 ratio till d25 post-AI and declined on d28 to 40 (Table 3). The high ratio occurs when P_4 is high relative to E_2 and this describes the classic situation of P_4 dominance. An embryo must secrete sufficient amounts of interferon-tau by d16 to prevent the regression of corpus luteum (Vasconcelos *et al*., 1997). It was also noticed that P_4/E_2 ratio was high on d0 (day of estrus and AI) in cows with early embryonic loss than cows diagnosed pregnant or cows with late embryonic loss (d25-40, Table 3).

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CONCEPTION RATE BASED ACCURACY OF DIFFERENT METHODS TO DIAGNOSE SUB-CLINICAL ENDOMETRITIS IN COWS

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ABSTRACT

The status of genitalia (through rectal palpation) and cervico-vaginal discharge at spontaneous estrus was examined in 140 clinically normal cows (Jersey and Jersey crossbred). Endometrial cytology (n=15), bacteriological culture analysis (n=140) and Whiteside test (n=114) used for the diagnosis of sub-clinical endometritis (SCE) in cows recorded the prevalence as 40.0, 58.8 and 55.0%, respectively. With respective methods, the conception rate in SCE positive cows was 40.0, 32.4 and 16.7% and in SCE negative cows was 48.6, 55.6 and 77.8%. Thus, based on conception rate, the endometrial cytology was the most effective test for the diagnosis of SCE.

Keywords: Bacteriological culture, Conception rate, Endometrial cytology, Endometritis, Whiteside test

INTRODUCTION

In repeat breeder cows, the high prevalence of sub-clinical endometritis (SCE) is hypothesized as the main etiological factor as most of the cases go undiagnosed (Parkinson, 2009). Due to the presence of polymorphonuclear (PMN) cells in the endometrial lumen, the SCE was first described as cytological endometritis (Gilbert *et al*., 1998), and was subsequently standardized based on the negative effects on reproductive performance (Madoz *et al*., 2014). The most reliable method for diagnosing SCE is cytobrush cytology (Barlund *et al.,* 2008). Despite the recognized negative effects of SCE on reproduction, the standardization of SCE diagnosis is not fully established (Pascottini *et al*., 2015). The lack of on-farm method to diagnose SCE leads to inability of farmers to routinely monitor SCE in commercial herds. In this study, attempts were made to record prevalence of SCE by using different diagnostic methods and the effect of SCE on bovine reproductive performance.

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MATERIALS AND METHODS

For this study, 140 clinically normal cows were selected based on the absence of abnormal discharge on external inspection. Apparently clear cervico-vaginal discharge samples were collected in a sterile screw capped vial containing sterile swabs. Subsequently, the cows were inseminated with frozen thawed semen (0.25 ml) and their reproductive performance was recorded.

For bacteriological culture analysis, the collected discharge samples (n=140) were inoculated in nutrient broth and incubated for 24-48 h and turbidity was observed. Whiteside test was conducted in 114 cows. One ml uterine discharge was mixed with one ml 5% sodium hydroxide solution in a test tube, heated upto boiling point and subsequently cooled in running tap water. The appearance of yellow color was positive indication of infection. Depending upon the intensity of color development, the degree of infection was classified; no color (absence of infection), mild yellow color change (mild infection), and intense yellow color (severe infection; Anilkumar and Devanathan, 1996).

Endometrial samples were collected by cytobrush in 15 cows. Sample must contain epithelial cells to

Table 1: Reproductive performance of non-infected and sub-clinical endometritic (SCE) cows based on different diagnostic methods.

Methods	SCE	Inseminated	Conception rate, %
Bacteriological culture, n=77	+	40	40.0
	-	37	48.6
Whiteside test, n=55	+	37	32.4
	-	18	55.6
Endometrial cytology, n=15	+	6	16.7
	-	9	77.8

confirm the correct site of collection. If no epithelial cells are seen, there is no assurance that the sample is taken from uterus. Slides for cytological examination were prepared by rolling the cytobrush on to a clean glass microscope slide, air dried and fixed in methanol for 15-20 min and stained with Giemsa stain for 45 min. The stained smears were examined at 100x by counting 100 inflammatory and other cell types. Cows with >5% PMN in the smear were regarded positive for SCE (Gilbert *et al*., 2005).

RESULTS AND DISCUSSION

In the present study, the inoculation of clear cervical mucus in nutrient broth indicated SCE in 55% cows, whereas, it was 58.8% on the basis of Whiteside test and 40% according to endometrial cytology. In a previous study, bacteriological culture of cervical mucus showed growth of aerobic organisms in 57.14% and SCE was diagnosed in 72% cows based on Whiteside test (Raja *et al*., 2012). Using the threshold of ≥5% PMN in endometrial cytology, the prevalence of SCE was higher in present study than a previous study with 13.8% prevalence (Pothmann *et al*., 2013).

In this study, following the collection of uterine discharge for the analysis of SCE, the cows were inseminated. Based on microbial growth, SCE positive cows exhibited 40% conception rate, whereas based on Whiteside test and endometrial cytology findings, 32.4% and 16.7% infected cows conceived (Table 1). The conception rate in non-SCE cows based on bacteriological, Whiteside and cytology test was 48.6, 55.6 and 77.8%, respectively (Table 1). Others recorded 5% and 47% CR after sampling cows with or without SCE (Salasel *et al*., 2010).

Thus, the comparison of diagnostic techniques indicated endometrial cytology as the best method to diagnose SCE because the CR was lowest in infected animals. In fact, neutrophils constitute the first defensive barrier against invading pathogenic organisms, resulting in an increase in the PMN cell population within the uterine lumen (Herath *et al*., 2009). Once a pathogen comes into contact with endometrium, the endometrium is stimulated to produce cytokines and chemokines that attract immune cells, in particular PMN cells, into the uterus (Galvao *et al*., 2011).

In conclusion, uterine cytology was the most accurate method as compared to Whiteside test and bacteriological culture to diagnose sub-clinical endometritis in cows.

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EFFECT OF MELATONIN ON NUCLEAR MATURATION OF CAPRINE OOCYTES

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ABSTRACT

Melatonin at the concentrations of 0, 5, 10, 20, 30, 40, and 50 ng/ml was used in maturation medium to assess the impact on *in vitro* maturation of caprine oocytes. The oocytes from slaughter house derived ovaries were subjected to *in vitro* maturation at 38.5°C with 5%CO_2. After culture for 27h, over 90% COCs had full cumulus cell expansion. The degree of cumulus cell expansion remained similar ($p>0.05$) on increasing melatonin concentration, however, oocytes incubated in 30 and 50 ng/ml melatonin containing maturation media for 27 h, result in 80% and 18.9% nuclear maturation rate, respectively, which were different ($p<0.05$) from control (45.3%). The nuclear maturation rate decreased ($p<0.05$) when melatonin concentration was increased from 30 to 40 and 50 ng/ml (80% *vs.* 28.3% and 18.9%, respectively). In conclusion, melatonin improved nuclear maturation of caprine oocytes at 30 ng/ml, whereas a high concentration of melatonin may affect caprine oocytes meiotic maturation at metaphase-II stage and can be toxic for caprine oocytes.

Keywords: Caprine, *in-vitro* maturation, Melatonin, Metaphase II, Oocytes.

INTRODUCTION

The manipulation of gametes and embryo increases the risk of exposure of these cells to high levels of reactive oxygen species (ROS) (Agrawal *et al*., 2006). Increased oxidative stress damages mitochondria and consequently impairs ATP production, and hampers meiotic and mitotic spindles formation in growing oocyte. It also destroys oocyte cell membrane lipids and DNA and progresses apoptosis quickly to inhibit fertilization (Kowaltowski *et al*., 1999).

Oxidative stress can be decreased by the presence of an antioxidant or radical scavenger in *in vitro* culture medium. Melatonin (MT; N-acetyl-5-methoxytryptamine) was successfully tested for promoting *in vitro* embryo development in many species including bovine (Manjunatha *et al*., 2009) and porcine (Kang *et al*., 2008). However, the effect of melatonin in maturation media for *in vitro* maturation of caprine oocytes has not been evaluated. Therefore, the present study was undertaken to investigate the optimum concentration of melatonin required for *in vitro* maturation of caprine oocytes.

MATERIALS AND METHODS

During spring season (February - April), goat ovaries (n=184) were collected from a local abattoir and were transported within 4h to laboratory in warm saline (35-37^{0}C), containing100 IU penicillin-G and 100 µg streptomycin sulfate per ml. Oocytes were retrieved by slicing goat ovaries and the recovered oocytes were graded (Kharche *et al.,* 2008).

Selected cumulus oocyte complexes (COCs) were washed two or three times in Oocyte Holding Medium (OHM) (TCM-199 medium, EGS 10%, Sodium Pyruvate 0.25 mM, Gentamicin 50 µg/ml, L-glutamine 100 µg/ml, BSA 3 mg/ml) and subsequently 8 to 10 times in 50-100 µl drops of oocyte maturation media supplemented with TCM-199 with 10% FBS, 10% follicular fluid, sodium pyruvate 0.25 mM, L-glutamine 100 µg/ml, LH 10µg/ml, FSH 5 µg/ml, estradiol-17β 1

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µg/ml, EGF 10ng/ml, BSA 3 mg/ml and gentamicin 50 µg/ml and allowed for maturation in 50 µl droplets of maturation media covered with sterile mineral oil for 27h in humidified atmosphere of 5% CO_2 at 38.5^{0}C in CO_2 incubator.

Matured oocytes (Fig. 1) were randomly divided into different treatment groups of maturation media on the basis of concentrations of melatonin added viz. group 1 (control, n=172), group 2 (5 ng/ml, n=120), group 3 (10 ng/ml, n=105), group 4 (20 ng/ml, n=125), group 5 (30 ng/ml, n=105), group 6 (40 ng/ml, n=113) and group 7 (50 ng/ml, n=111).

After 27 h maturation, oocytes were stripped off their cumulus cells by gentle pipetting for 1 min in 0.1% hyaluronidase enzyme. Denuded oocytes were selected and washed in PBS (1X) followed by fixation with Para-formaldehyde for 10 min. Oocytes were stained with Hoechst33342 dye (1µL/mL dissolved in DMSO was stored at 2–6° C, protected from light) for 30 min in dark. Therafter, oocytes were washed with 1X PBS and evaluated under an Inverted phase-contrast microscope. Nuclear stages were distinguished by the morphology of chromatin material (Hewitt *et al*., 1998; Yadav *et al*., 2013). Oocytes with second metaphase plate (two chromatin spot) and first polar body were classified as mature phase of second meiotic cell division (Fig. 2).

The maturation rates between different treatment groups were compared using the Chi-square test. The level of significance was recorded at the 5% level of confidence.

RESULTS AND DISCUSSION

From slaughtered goat ovaries, by slicing technique, the oocyte recovery rate was 3.94. The nuclear stages of matured oocytes were distinguished by the morphology of chromatin material (Hewitt *et al*., 1998 and Yadav *et al*., 2013). Oocytes with first polar body or two chromatin spots were classified as mature phase of second meiotic cell division (MII).

Oocytes that show polar body or two chromatin spots were considered as matured oocytes (Fig. 1). The rate of oocytes maturation to MII stage was higher ($p<0.05$) with 30 ng/ml MT (80%), 20 ng/ml MT (68%), and 10 ng/ml MT (55.2%) as compared to control (45.3%, Table 1), possibly due to antioxidant role of melatonin. However, the oocyte maturation rate lowered in 40 ng/ml MT (28.3%) and 50 ng/ml MT (18.9%, Table 1). This could be due to toxic effect of melatonin at higher concentration, thus leading to lowering maturation rate and resulting in degeneration of oocytes (Tamura *et al*., 2009).

Melatonin (N-acetyl-5-methoxytryptamine) directly destroys free radicals, indirectly stimulates antioxidant enzymes and inhibits peroxidation enzymes such as nitric oxide synthetase (Galano *et al*., 2011). Melatonin accelerates the action of maturation-inducing hormone on maturation-promoting factor and germinal vesicle breakdown of oocytes (Chattoraj *et al*., 2005). The

Table 1: Nuclear maturation rate of caprine oocytes at different Melatonin concentrations

Melatonin, ng/ml	Oocytes, n	Matured oocytes, n	Maturation, %
0	172	78^{a,c}	45.4±0.4
5	120	56^{a,c}	46.5±0.2
10	105	58^{a,b}	55.1±0.3
20	125	86^{a,b}	68.6±0.1
30	105	84^{b}	80.2±0.1
40	113	32^{c}	28.2±0.3
50	111	22^{c}	19.5±0.1

$^{a,b}p<0.05$

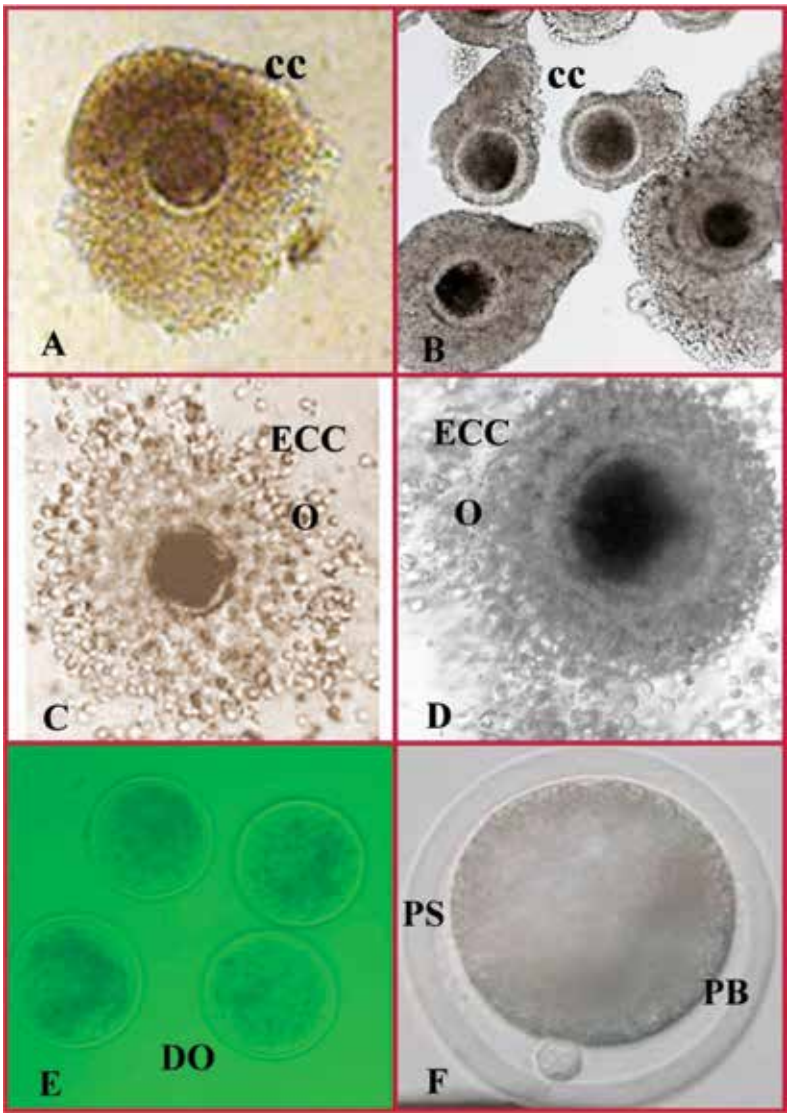

Fig. 1: Morphological maturation of caprine oocytes. A, B - Immature oocytes with compact cumulus cells (cc); C, D - Oocytes (O) with expanded cumulus cells (ECC) after *in vitro* maturation; E - Denuded oocytes (DO) after *in vitro* maturation; F - Matured oocytes with extruded polar body (PB) and peri-vitelline space (PS)

present study demonstrates that melatonin has a powerful antioxidative effect during IVM of bovine oocytes. In addition, the melatonin receptors were identified in granulosa cells, which suggest another possibility by which melatonin may take part in oocyte maturation.

The effect of different doses of melatonin on degree of nuclear maturation of oocytes investigated the optimal concentration for IVM of oocytes. The nuclear stages were identified as germinal vesicle stage (GV), germinal vesicle breakdown stage (GVBD), metaphase I stage (M I) and metaphase II stage (M II) with extruded polar body oocytes and Metaphase II plates were counted as mature (Fig. 2). The chromosomes in polar bodies with intact plasma membranes fluoresced blue. The observations from present study revealed

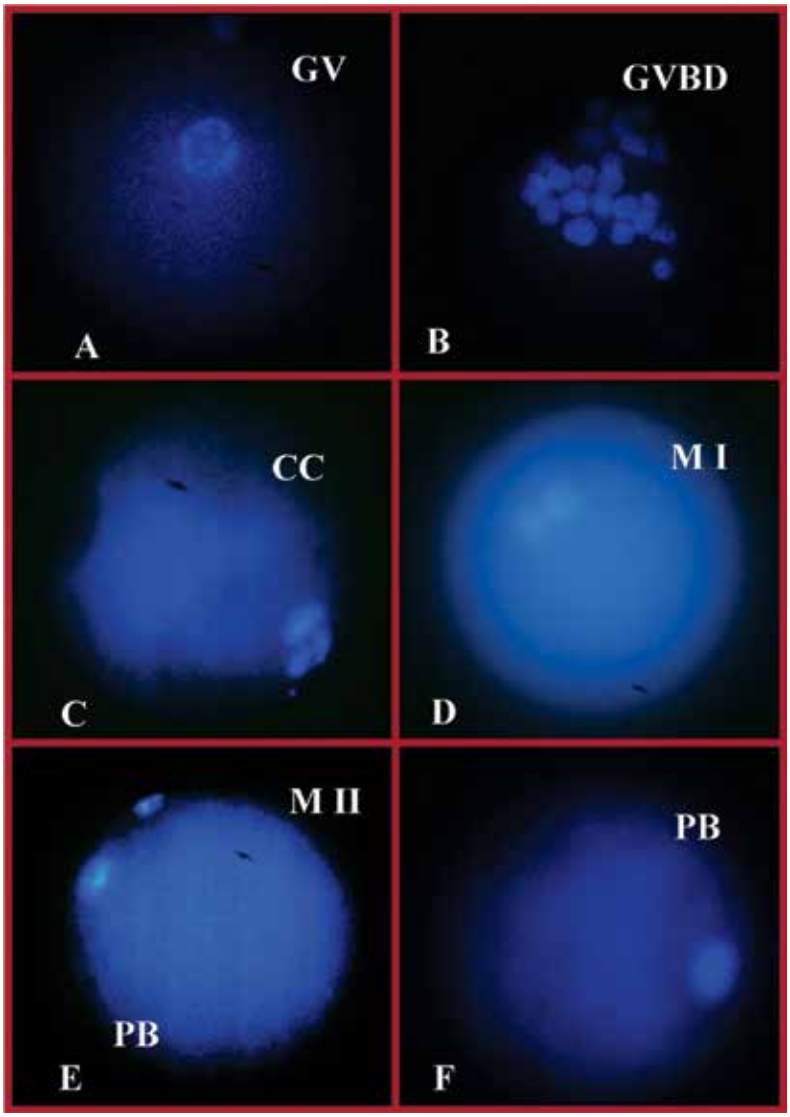

Fig. 2: Nuclear maturation of caprine oocytes. A - Oocyte showing germinal vesicle (GV) stage. Nuclear membrane is visible; B - Oocyte showing germinal vesicle break down stage (GVBD); C - Oocyte after GVBD with condensed chromatin mass (C); D - Oocyte with chromosomes arranged in equatorial plane at metaphase I (M I Stage); E, F - Oocytes showing metaphase II (M II stage) and extruded polar body (PB) under Hoechst staining (40X)

that all polar bodies had a sharply defined, smooth membrane and clear cytoplasm with chromosomes as scattered, stretched, or adherent to each other (Fig. 2).

In fact, the high concentrations of melatonin in follicular fluid (Brzezinski *et al.,* 1987), and the presence of receptors in granulose cells (Na *et al*., 2005), suggest that melatonin might be important to ovarian functions. The addition of melatonin to culture medium may increase the cumulus expansion and *in vitro* maturation of oocytes. The cumulus cells are known to play a crucial role during oocyte maturation. For acquiring developmental competence by oocyte *in vitro,* the cumulus cells during maturation are essential (Gordon, 2003). It was reported that 1 µM melatonin

reduces cumulus cells apoptosis by activating its receptors on cumulus cells (Na *et al*., 2005).

Thus, melatonin improved the nuclear maturation of caprine oocytes at 30 ng/ml, whereas a high concentration of affected *caprine* oocytes meiotic maturation at metaphase-II stage and can be toxic for caprine oocytes.

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ADDITION OF CHLOROQUINE DIPHOSPHATE OR ASCORBIC ACID IN JERSEY BULL SEMEN AND SUBSEQUENT EVALUATION WITH RESPECT TO POST-THAW INCUBATION TIME AND CONCEPTION RATE

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ABSTRACT

Total 36 ejaculates (6 from each Jersey bull) were used in the study and aliquots were added with Chloroquine diphosphate or Ascorbic acid or served as control. Semen straws from all the groups were analyzed at 0, 1 and 2 h post-thaw incubation at 37°C for percent viability, progressive motility, reaction to hypo-osmotic solution and acrosomal integrity. Following post-thaw incubation, an improvement ($p<0.05$) in all the evaluated semen parameters was recored in groups with semen additives compared to control. The conception rate was better ($p<0.05$) using semen fortified with ascorbic acid.

Keywords: Acrosomal integrity, Ascorbic acid, Chloroquine diphosphate, Conception rate, HOST

INTRODUCTION

Mammalian spermatozoa are extremely sensitive to oxidative damage, *in-vivo* as well as *in-vitro*. The process of peroxidation induces structural alterations, particularly in the acrosomal region, a fast and irreversible loss of motility, a deep change in metabolism and a high rate of intracellular components release (Cecil and Baskt, 1993). Membrane stabilizers and antioxidants have beneficial effect on the membrane integrity and biofunctional activity of spermatozoa. Ascorbic acid, a biologically active reducing agent, restores fertility possibly by the reduction of anti-agglutination factor on sperm membrane from inactive form to active form (Lindahl, 1966). Chloroquine diphosphate as membrane stabilizer was used earlier in preservation of buffalo semen (Kumar, 1992). The success of cryopreservation depends largely on the specific susceptibility of sperm cells to low temperature. The overall impact is seen in fertility in terms of lowered conception than with the fresh semen.

The present study investigated the impact of semen additives (Chloroquine diphosphate and Ascorbic acid) on routine semen evaluation parameters with respect to post-incubation time and conception rate.

MATERIALS AND METHODS

The study was conducted on 36 ejaculates (6 from each bull) collected twice a week from each of six apparently healthy purebred Jersey breeding bulls (age, 2.5-8.0 yr), maintained at Sperm Station, Palampur, Himachal Pradesh, India (32.6^{O}N, 76.3^{O}E, altitude 1290.8 m). After initial evaluation, the neat semen extended in TRIS-based extender was divided into three aliquots (10 ml diluted semen) viz. control (G_1), Chloroquine diphosphate @ 10^{-5}M concentration (G_2) or Ascorbic acid @ 0.02% concentration (G_3). All the semen dilution, extension and modified extension procedures were carried out at 37^{o}C with 80M spermatozoa/ml of diluted semen. Tested semen samples were filled in 0.25 ml French mini plastic straws and these were frozen as per the standard procedures. Semen straws from all the groups were thawed at 37^{o}C for 30 second and were evaluated at 0, 1 and 2 h post-thaw for percent viability, progressive motility, reaction to 150 mOsmol hypo-osmotic solution, and acrosomal integrity. For fertility trials, 55 cows were inseminated

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Table 1: Relationship between post-thaw incubation time with semen evaluation parameters following the addition of semen additives. G1, Control; G2, Chloroquine Diphosphate; G3, Ascorbic acid

Group	Correlation Coefficient	Regression Estimate	Regression Equation
Live Sperms, %			
G_1	-0.72**	-6.34±0.81	y = 53.85±1.04–6.34x±0.81
G_2	-0.73**	-6.67±0.81	y = 57.28±1.11–6.67x±0.81
G_3	-0.75**	-6.62±0.17	y = 57.98±0.93–6.62x±0.17
Progressive Motility, %			
G_1	-0.80**	-9.65±0.71	y = 47.84±0.91–9.65x±0.71
G_2	-0.76**	-9.06±0.72	y = 50.10±0.93–9.06x±0.72
G_3	-0.83**	-9.31±0.61	y = 51.24±0.80-9.31x±0.61
HOST, %			
G_1	-0.78**	-7.71±0.59	y =40.74±0.77–7.71x±0.59
G_2	-0.81**	-7.38±0.53	y =46.18±0.63–7.38x±0.53
G_3	-0.75**	-7.53±0.65	y =45.53±0.84–7.53x±0.65
Acrosomal Integrity, %			
G_1	-0.78**	-7.82±0.61	y =67.78±0.79–7.82x±0.61
G_2	-0.69**	-5.96±0.59	y =68.25±0.76–5.96x ±0.59
G_3	-0.79**	-6.63±0.49	y =70.01±0.64–6.63x±0.49

**p<0.01

with straws of each group The data were analyzed by SPSS® 20 level version for windows.

RESULTS AND DISCUSSION

All the semen quality assessment traits viz. live sperms, progressive motility, HOST reactive spermatozoa and acrosomal integrity were negatively and significantly (p<0.05) correlated with the post-thaw incubation time (Table 1), as observed earlier (Sharma *et al.*, 2012; Rastegarnia *et al.*, 2013). Furthermore, the post-thaw deterioration of semen quality was comparatively less in semen with additives in comparison to unfortified one (Table 1). Out of 165 cows inseminated (n=55 in each group), the conception rate was 45.5, 56.4 and 65.5% in cows of control, Chloroquine diphosphate and Ascorbic acid group, respectively. Thus, ascorbic acid as an anti-oxidant improved (p<0.05) the post-thaw quality of frozen semen of Jersey bulls. Higher conception rates with no statistical difference were also observed in the semen preserved with Chloroquine diphosphate in buffaloes (Singh *et al*., 2000). The decline in progressive motility observed in the present study after storage may be associated with decrease in live-dead sperm ratio and gradual exhaustion of some vital endogenous reserves in a highly differentiated cell which has lost its capacity for protein synthesis (Kumar, 2007).

In brief, it can be concluded that semen parameters had negative correlation with post-thaw incubation time, however, post-thaw deterioration of the semen quality was comparatively less in semen with additives and the conception rate was better following the use of fortified semen.

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EFFECT OF TWO DIFFERENT PERMEABLE CRYOPROTECTANTS ON FREEZABILITY OF EXOTIC STALLION AND JACK SEMEN

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ABSTRACT

Semen was collected from the exotic stallions and exotic jacks (n=5 each) using an artificial vagina. The fresh semen was treated with primary extender and centrifuged to remove seminal plasma. Sperm pellet obtained after centrifugation was equally divided to receive secondary extender containing same amount (5%) of different permeable cryoprotectants, either glycerol (Gly) or dimethyl formamide (DMF) before submitting for cryopreservation. Various sperm parameters were assessed at pre-freeze and post-thaw stage which revealed differences ($p<0.05$) in motility, liveability, plasma membrane integrity and acrosome integrity of stallion as well as Jack semen cryopreserved with DMF or Gly. A variation ($p<0.05$) existed between individual stallions and jacks extended either with DMF or Gly. In brief, cryoprotectant DMF for stallion and Gly for jack semen were suitable for retaining the plasma and acrosome integrity of spermatozoa.

Keywords: Cryopreservation, Di-Methyl Formamide, Glycerol, Jack, Semen, Stallion

INTRODUCTION

Equine semen is a one of the most difficult in industry to cryopreserve efficiently without causing damage to membrane or apoptosis. The successful use of cryopreserved sperm largely depends on cryosurvival rates, which show large variation among species and individuals of the same species (Vidament *et al*., 2009; Wu *et al*., 2015). In fact, only 20% of fertile stallions produce sperms that survive the freezing and thawing processes (Tischner, 1979). Glycerol (Gly) is a preliminary cryoprotectant that has not only beneficial cryoprotective effects (Hoffman *et al*., 2011), but also toxic effects on spermatozoa (Alvarenga *et al*., 2005), despite with contraceptive effects in mare (Vidament *et al*., 2009). Glycerol lowered the fertility of equine semen when included in extenders for fresh, cooled, and frozen semen (Wu *et al*., 2015). Other cryopritectant like Dimethyl formamide (DMF) were less toxic and yielded similar or superior results as compared to glycerol (Alvarenga *et al*., 2005). Therefore, the present study aimed to assess the effects of two permeable cryoprotectants viz. Glycerol (GLY) and Dimethyl Formamide (DMF) on the freezability of exotic stallion and jack semen.

MATERIALS AND METHODS

Fiive healthy exotic (Thoroughbred) stallions and five exotic Jacks (Poitou breed), aged 6-10 yr, maintained in well-ventilated boxes on a standard diet (5 kg concentrate with mineral mixture, salt and 15 kg fodder - green: dry in 3:1 ratio) and *ad lib* fresh drinking water were used in the present study during the breeding season.

The semen from all the animals were collected using artificial vagina (Colorado model) equipped with a disposable liner as per the standard method (Talluri *et al*., 2017). Semen collection, evaluation and processing for freezing were done according to described methods (Talluri *et al*., 2017). Immediately after semen collection, seminal parameters like appearance, volume, colour, consistency, pH were recorded by visual observation. The gel portion of fresh semen was sieved through a sterilised gauze filter and volume of total and gel free

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semen was calculated visually through graduated sterile semen collection bottle. For calculating total gel volume, the gel free semen volume was deducted from fresh semen collected before sieving. After performing macroscopic and microscopic evaluation of fresh semen, the semen samples were processed, extended and frozen according to the described methods (Talluri *et al*., 2012a).

Semen samples having progressive motility >60% were processed for cryopreservation. Gel free semen was mixed with modified Citrate-EDTA primary extender in the ratio of 1:1 and centrifuged at 550 g for 3 min. The supernatant was discarded and sperm pellet was extended with Glucose-EDTA-lactose secondary extender having cryoprotectant agents either 5% GLY or 5% DMF. The diluted semen (100×10^6 sperm cells/ml) was kept in semen cooling cabinet at 4ºC for 2 h as equilibration period. Semen samples were again assessed for pre-freeze seminal characteristics. Equilibrated semen was manually filled in 0.5 ml straws using vacuum pump. The straws were sealed using PVC powder and were cooled for 30 min at 4ºC. The straws were spread over freezing racks, 4 cm above liquid nitrogen (LN_2) in a traditional styrofoam box for exposure to LN_2 vapours for 10-12 min and then plunged directly into LN_2. Later, the semen straws were transferred to LN_2 cryocans. The microscopic evaluation of frozen thawed sperm was done at least 24 h after storage. The straws were thawed in 37ºC water bath for 30 sec. Each frozen thawed semen sample was evaluated for determining the post-thaw motility, live and dead percentage, plasma membrane integrity through hypo-osmotic swelling (HOS) test and acrosome integrity as per the standard procedures.

As repeated collection of semen was done on same stallion/jack at different time intervals, a repeated measure ANOVA was done to partition the variability attributable to differences between treatments and individual variation among stallion/jack/subjects in treatment groups. Statistical analyses were performed using the SPSS 20.0 statistical software package. One-way ANOVA was used to test statistical differences between different treatment groups. Pair wise comparisons (or *post hoc* test) were performed using the T-method (Tukey's honestly significant difference method).

RESULTS AND DISCUSSION

The fresh semen collected from exotic stallions and jacks was white to creamy white and consistency was variably thick and viscous as observed earlier (Talluri *et al*., 2018). The fresh semen volume collected from stallions ranged from 25-125 ml and in jacks from 45-83 ml (Table 1). The total volume of stallion semen varies between 30-250 ml (Ricketts, 1993). In the current study, average total semen volume, gel free semen and gel in semen was recorded less than reported for indigenous stallions (Talluri *et al*., 2012a and 2012b). The pH of stallion semen ranged from 6.92-7.21 and of jacks was 6.52-7.10 (Table 1). The pH range was in correlation with earlier reports for stallions (Talluri *et al*., 2018) and Jacks (Rabindra Kumar *et al*., 2018).

The sperm concentration in fresh semen was $185.7\text{-}294.7\times10^6$ ml^{-1} in stallions and $281.7\text{-}341.1\times10^6$ ml^{-1} in jacks. with a mean of $318.29\pm16.29\times10^6$ ml^{-1} (Table 1). The variation in sperm concentration between individual stallions and jacks was simular ($p<0.05$), however, between stallions and jacks was different ($p<0.05$). The sperm concentration for exotic stallions and jacks observed in this study was higher compared to earlier reports for Stallions (Talluri *et al*., 20018) and jacks (Rabindra Kumar *et al*., 2018). Total sperm motility in gel fractioned semen was 79.3-88.2% in stallions and was 80.9 to 91.1% for jacks (Table 1), which was higher than observed for Marwari stallions (Pal *et al*., 2009) and Poitou jacks (Rabindra Kumar *et al*., 2018). The progressive sperm motility in gel free semen was 70.2-79.9% for stallions and 76.3-84.7% for jacks (Table 1). The stallion semen exhibiting >60% progressive sperm motility can be considered appropriate for cryopreservation.

Table 1: Fresh seminal characteristics of exotic Stallion and Poitou Jack (n=40 each).

Seminal attribute	Stallion	Jack
Total Volume, ml	45±6.7	53.8±8.3
Gel Volume, ml	9±1.18	9.6±0.81
Final volume, ml	36±5.7	45.2±8.3
pH	7.03±0.01	6.82±0.06
Sperm Concentration, $x10^6$	205.4±12.6	389.0±15.5
Total Initial Motility, ml	81±5.3	85±2.7
Progressive Motility, ml	74.7±1.70	80±2.73

A major difference was observed in pre-freeze and post-thaw motility of stallion spermatozoa cryopreserved with either DMF or Gly (Fig. 1). The stallion semen extended with DMF had better ($p<0.05$) progressive motility compared to Gly both at pre-freeze and post-thaw stages. Furthermore, at pre-freeze and post-thaw stages, a difference ($p<0.05$) existed in sperm motility between individual stallions treated with DMF or Gly (Fig. 1). In case of jacks, the semen cryopreserved with Gly had better ($p<0.05$) motility both at pre-freeze and post-thaw stages. The jack semen treated with Gly exhibited superior ($p<0.05$) motility over DMF or Gly treated stallion semen with at both stages (Fig. 1).

At post-thaw stage, the liveability was better ($p<0.05$) for stallion and jack semen cryopreserved with DMF but not for semen extended with Gly (Fig. 1). The functional integrity of plasma membrane as determined by HOS test was different ($p<0.05$) in spermatozoa extended with DMF or Gly at pre-freeze stage but not the post-thawed stallion semen. The jack semen extended with Gly had good plasma membrane integrity than semen extended with DMF and the same was *vice versa* for the stallion semen (Fig. 1). A highly significant difference ($p<0.05$) was observed in acrosome integrity of stallion spermatozoa extended with DMF or Gly at pre-freeze stages only.

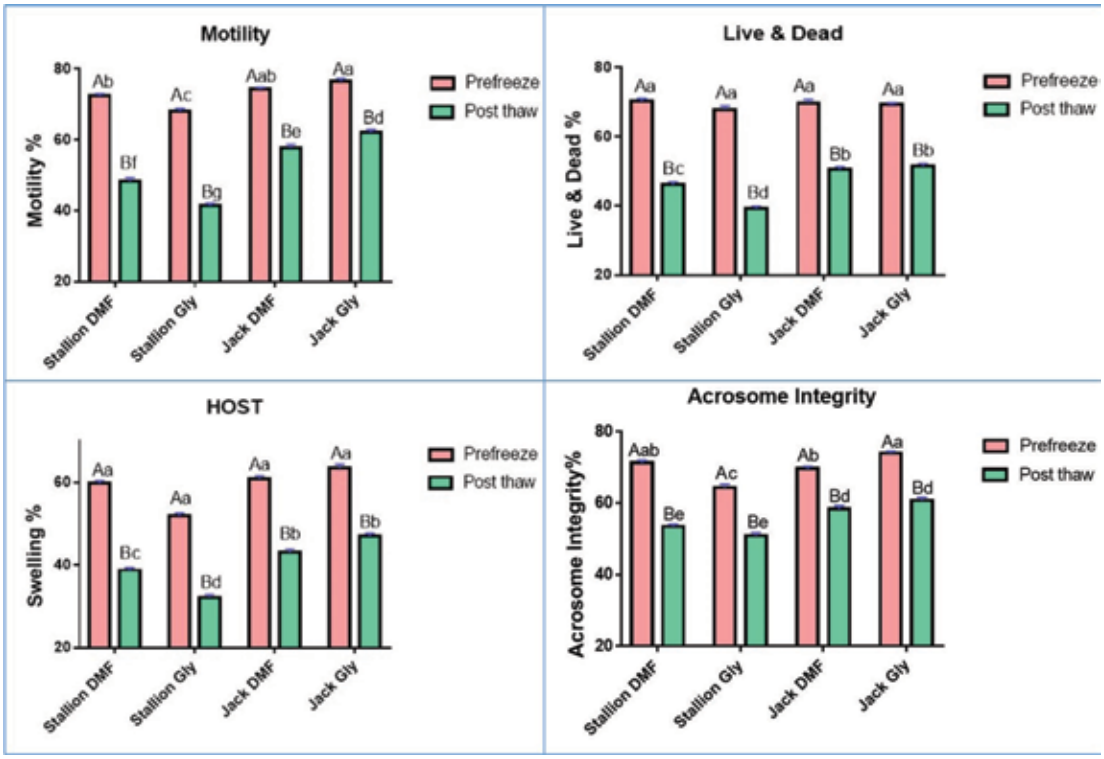

Fig. 1: Pre-freeze and post-thaw seminal characteristics of exotic Stallion and Jacks (n= 40 each group). ^{A,B}p<0.05 - within a group (between pre-freeze and post thaw). $^{a-g}$p<0.05 - across the groups.

The stallion semen treated with DMF had higher ($p<0.05$) percentage of sperms with acrosome integrity than that of Gly at both pre-freeze and post-thaw stages. Furthermore, the difference ($p<0.05$) in acrosome integrity was observed between individual stallion semen treated with DMF or Gly at post-thaw stage only (Fig. 1). In jacks, the semen extended with either DMF or Gly had difference in acrosome integrity at post-thaw stage ($p<0.05$), with the semen extended with Gly had higher acrosome integrity than DMF (Fig. 1). The freezability of semen from exotic horses and exotic jacks was 90% (20 ejaculates out of total 22 ejaculates) and 95% (21 ejaculates out of total 22 ejaculates), respectively.

In brief, the stallion and jack spermatozoa may be damaged by glycerol, but the toxic effect of glycerol is more obvious for the stallions. Thus, DMF can be used as an alternative to Gly as cryoprotectants for stallions and Gly can be used for jack semen to obtain better post-thaw motility and integrity of sperm membranes.

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EFFICACY OF GARLIC EXTRACT±ASHWAGANDHA FOR THE TREATMENT OF INFECTIOUS REPEAT BREEDING IN CATTLE

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ABSTRACT

The present research work was conducted to evaluate the efficacy of Garlic extract intrauterine (IU) *versus* Garlic extract (IU) plus Ashwagandha powder (oral) for the treatment of fresh (repeated ≤7 times) and chronic (repeated >7 times) infectious repeat breeding in cattle, respectively. Uterine infection was diagnosed by pH estimation, PMN cell count and White Side Test of cervico-vaginal mucus in both fresh and chronic cases while Immunoglobulin flocculation test with serum in chronic cases only. This indicated 92.8% cows having alkaline pH, PMN cell count >7% and 100% positive for White Side Test and increased immunoglobulin level in blood. Total 36 cows affected with endometritis were divided into three groups to undertake treatment on day of estrus *viz.* Group-I consisting of 12 fresh cases were treated with Garlic extract @10 µg in 30 ml PBS by intrauterine route once; Group-II consisting of 12 chronic cases were treated with Garlic extract as per Group-I plus Ashwagandha powder @ 15 gm with concentrates per oral for 5 days; and Group-III consisting of 12 cows as control were treated with placebo @ 30 ml PBS intrauterine. The clinical recovery of cows was confirmed by normal pH, negative white site test and PMN cell count <5% in cervico-vaginal mucus at subsequent estrus. Immunoglobulin flocculation test revealed raised immunoglobulin level in recovered cases of chronic endometritis. The recovered cows were inseminated with frozen-thawed semen at mid stage of estrus and pregnancy was confirmed at 50-60 day post insemination. The results of present investigation revealed highest recovery rate (83.3%) in group-I, whereas the highest conception rate (66.7%) and pregnancy rate (55.6%) was observed in group-II, thus suggesting the use of Garlic extract alomg with Ashwagandha for infectious repeat breeder cattle.

Keywords: Ashwagandha, Cows, Garlic extract, Infectious, Repeat breeder

INTRODUCTION

The success of dairy farming lies in ensuring the optimal reproductive efficiency as reproductive failure results in progressive economic losses. Among various reproductive disorders, repeat breeding is one of the major gynaecological problems (Kumar *et al*., 2017). Uterine infection implies adherence of pathogenic organisms to uterine mucosa and followed by colonization, penetration of the endometrial epithelium and release of bacterial toxins setting up uterine inflammation. Incidence of clinical and sub-clinical endometritis in cows is 12 % and 29.7%, respectively (Kumar *et al*., 2018). Several approaches including antibiotics, antiseptics and hormonal therapies were employed to treat repeat breeding cows with endometritis. However, there are certain limitations in the use of antibiotics like drug resistance, inhibition of normal uterine defense mechanisms and residual effect of antibiotics and hormones in the milk and meat (Rahi *et al*., 2013). Various herbs possess antibacterial, antiviral, antifungal, antioxidant and immunomodulatory properties ensuring prevention and cure of several diseases and disorders without side effects. Hence, different herbs were used as a source of valuable medicines globally (Gebreyohannes *et al*., 2013). Therefore, in the present study, herbal Garlic and Ashwagandha preparations were used for the treatment of repeat breeding due to infectious endometritis in cows.

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MATERIALS AND METHODS

The study was carried out in 36 infectious repeat breeder cows under field and farms. Infectious repeat breeding cows were selected on the basis of history and breeding records (repeated ≥3 times), results from laboratory investigation of cervico-vaginal mucus (CVM) *viz.* alkaline pH, positive white site test, presence of white flakes (Popov, 1969) and increased PMN cell count (>7%) in endometrial cytology smear and immunoglobuline flocculation test of serum (Deshpande *et al.,* 1991). Selected 36 endometritic cows were equally divided into 3 groups. Group-I repeat breeder cows (n=12) repeated seven times or less were administered with single intra uterine infusion of 10 mcg *Methanolic Garlic extract* dissolved in 30 ml PBS during mid-estrus stage. Group-II repeat breeder cows (n=12) repeated >7 times were administered with single intrauterine infusion of 10 mcg *Methanolic Garlic extract* as per group-I and 15 gm Ashwagandha powder in concentrate was fed additionally once daily for 5 days and Group-III repeat breeder cows (n=12) were administered placebo treatment @ 30 ml PBS intrauterine during mid estrus and were inseminated at next estrus only. The CVM and blood samples were collected from test animals at estrus before and after treatment to evaluate the treatment efficacy. The serum was separated and stored at 20^0C till laboratory investigation.

Methanolic Garlic extract (20% W/V) was prepared in advance by adding 200 gm Garlic seeds pest in 100% methanol to make final volume 1000 ml in a conical flask. The solution was kept at room temperature for 48 h with constant shaking followed by sieving with muslin cloth and then filtration through ordinary filter paper. The filtrate was poured in wide stainless-steel plate and allowed to evaporate under room temperature till the formation of semi-solid pest which took around one month. From the prepared Garlic pest, each dose was prepared by dissolving 10 mcg pests in 5 ml PBS as stock solution. Thus, the prepared stock doses were stored in refrigeration temperature (4^0C). Transportation of doses to field was attempted in proper thermos flask on ice packs. Each dose was reconstituted with addition of 25 ml PBS for administration through intrauterine route. Ashwagandha powder available in market was used for therapeutic purpose and the powder was fed in concentrate @ 15 gm/day to the chronic cases for 5 days as an immuno-booster.

The clinical recovery of cows was confirmed by normal pH, negative white site test and PMN cell count <5% at subsequent oestrus. The recovered cows were inseminated with frozen-thawed semen at mid stage of estrus. Conception was ensured by non-return of estrus in subsequent cycle. Pregnancy was confirmed by trans-rectal palpation after 50-60 days of insemination. The data was statistically analyzed using analysis of variance (ANOVA) to find out the significant differences of mean values (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The present study was conducted to evaluate the antimicrobial property of Garlic and immunomodulatory effect of Ashwagandha herbs. Uterine infection in repeat breeder cows was diagnosed on the basis of pH estimation and White Side Test of cervico-vaginal mucus, uterine cytology of endometrial contents and Immunoglobulin Flocculation Test in serum. The diagnosis was made on the basis of cumulative results of all tests and no single test result was considered.

The pH observations in infectious repeat breeder cows were recorded as mildly acidic in 2.8%, normal in 13.9%, moderately alkaline in 61.1% and highly alkaline in 22.2% cases. A highly significant ($p<0.05$) number of cases were carrying pH from 8.1 to 8.5. White Side Test showed variable grade of severity of endometritis viz. 27.8% mild, 36.1% moderate and 19.5% severe. Endometrial cytology revealed PMN cell count as >5% in all selected cases. The PMN cell count ranged between 05-18% with an average of 9.8±0.3% and, thus diagnosed as subclinical stage

of endometritis (Sheldon *et al*., 2009; Bajaj *et al*., 2017). The average immunoglobulin concentration in endometritic cows was 6.9-7.0 mg/ml in 24 cases.

On treatment of Garlic extract by intrauterine route in infectious repeat breeding cows from Group-I, 10 cows (83.3%) responded to treatment against nil response in control group. Efficacy of Garlic extract was studied and reported previously with variable recovery rate from 50-100% in cases of infectious repeat breeder cows (Sarkar *et al*., 2006; Rahi *et al*., 2014; Yildiz, 2016; Kumar *et al*., 2018; Sharma *et al*., 2018). However, the current findings regarding recovery rate as 83.3% by garlic extract corroborate with others (Kumar *et al*., 2018; Sharma *et al*., 2018).

Mean pH value before treatment was 8.3±0.1 which changed to 7.3±0.1 in recovered cows after treatment; however, the same remained almost similar in non-recovered (8.4±0.0). The pH value of CVM was 7.8±0.2 before treatment which slightly increased to 8.4±0.1 after treatments in control cases which may be due to progression of uterine infection in untreated animals. Negative White Side Test was confirmed in all responded cases, but it remained positive in non-recovered and control group. Average PMN cell count was recorded as 9.4±0.5% before treatment, which reduced ($p<0.05$) to 2.9±0.3 in recovered cows, whereas, the same decreased ($p>0.05$) to 8.0±0.0 in non-recovered cows after treatment.

Conception rate was recorded as 60.0% in inseminated (only recovered) cows after Garlic extract treatment in the present study, which was slightly higher than earlier findings (Sarkar *et al*., 2006; Kumar *et al*., 2011; Rahi *et al*., 2014; Yildiz, 2016; Sharma *et al*., 2018; Kumar *et al*., 2018) who reported same (range: 50.0-52.4%). Further, the pregnancy was confirmed by trans-rectal palpation after 50-60 days of insemination and it was recorded as 50.0% as against nil pregnancy in control group.

Endometritis is basically caused by non-specific bacterial agents and moreover the infection is mostly undulant. Mixed infection evident in endometritis indicates body stress and lowered immune status. Garlic is natural protection even against physiological threats like oxidative stress, cardiovascular complexities and immune dysfunction. Garlic was recommended for immune-modulatory effect as it increases T-lymphocyte blastogenesis and phagocytosis with modulation of cytokine production (Mukherjee *et al*., 2014).

Combined treatment of Garlic extract with Ashwagandha in infectious repeat breeder cows from Group-II showed 75% recovery rate as against nil recovery in control group. Conception rate was recorded as 66.7% in inseminated cows. Subsequently, pregnancy rate was recorded as 55.6% on per-rectal palpation as against nil pregnancy in control group. It was observed that similar treatment was employed earlier (Rahi *et al.,* 2014), who reported 75% recovery rate and 62.5% conception rate in endometritis cows treated with Garlic extract IU plus Ashwagandha powder orally. However, they found higher recovery (87.5%) and conception (75.0%) rate when both Garlic extract and Ashwagandha were administered through intrauterine route.

Table 1: Mean pH and PMN cell count before and after treatment in infectious repeat breeder cows.

Group	Before treatment		After treatment			
			Recovered		Non-recovered	
	pH	PMN, %	pH	PMN, %	pH	PMN, %
Garlic Extract	8.3±0.1[a]	9.4±0.5[c]	7.3±0.1[b]	2.9±0.3[d]	8.4±0.0	8.0±0.0
Garlic + Ashwagandha	8.5±.01[a]	10.0±0.3[c]	7.4±0.1[b]	4.7±0.3[d]	8.2±0.0	8.5±0.0
Control	7.8±0.2	9.9±0.4	-	-	8.4±0.1	9.7±0.5

[a,b,c,d]$p<0.05$

The mean pH value of CVM reduced from 8.5±0.1 to 7.4±0.1 in recovered cows whereas, remained same (8.2±0.0) in non-recovered cows after treatments. WST became negative in all recovered cases while remained positive in non-recovered cases on subsequent estrus after treatment. Average PMN cell count was 10±0.3% before treatment in this group, which reduced to 4.7±0.3% ($p<0.05$) in recovered cases, whereas, reduced (8.5±0.0%; $p>0.05$) in non-recovered cows. The mean immunoglobulin level (mg/ml) in group-II increased from 6.8±0.4 to 12.3±0.9 after treatment. No report citing immunoglobuline flocculation test results could be traced from the literature for comparison of the current findings in infectious repeat breeding.

In conclusion, the study showed that although bare Garlic extract yielded higher recovery rate (83.3%), however the final pregnancy rate on treated basis was similar for both treatment protocols irrespective of chronic or fresh repeat breeding. Statistically, recovery (83.3 *vs.* 75.0%), conception (60.0 *vs.* 66.7%) and pregnancy rate (50.0 *vs.* 55.6%) in Garlic extract therapy *vs.* combination therapy differed ($p>0.05$) in endometritis cows.

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EFFECT OF OXYTOCIN, $PGF_{2\alpha}$ AND GnRH ON UTERINE INVOLUTION AND POSTPARTUM FERTILITY IN MURRAH BUFFALOES SUBJECTED TO FETOTOMY

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ABSTRACT

Out of dystocia affected Murrah buffaloes subjected to fetotomy operation (n=40), one group of 20 buffaloes was administered a) Oxytocin (50IU I/M) immediately and 4h after fetotomy, b) Prostaglandin $F_{2\alpha}$ (500µg I/M on d7 and d21 postpartum), and c) GnRH (buserlin acetate @ 20µg I/M) on day 28 postpartum. The second group of 20 animals subjected to fetotomy were not administered any test medication. The third group consisting of 10 normally calving buffalo served as control. The buffaloes of frist group had improved uterine health, ovarian rebound and became pregnant in lesser time than others. In brief, administration of ecbolics and GnRH in early postpartum period hastens uterine involution and improves reproductive efficiency of dystocia affected buffaloes.

Key words: Buffalo, Ecbolics, Fetotomy, GnRH, Involution, $PGF_{2\alpha}$, Postpartum

INTRODUCTION

During postpartum period, the important events include uterine involution, regeneration of endometrium, elimination of bacterial contamination of uterus and the return of ovarian cyclical activity (Peter *et al.,* 1987). Dystocia leads to uterine contamination and affects involution and subsequently fertility. Thus, dystocia affected buffaloes subjected to fetotomy were administered uterine ecbolics like oxytocin and $PGF_{2\alpha}$ and GnRH postpartum to augment the process of involution and revival of ovarian cyclicity.

MATERIALS AND METHODS

In 40 Murrah buffaloes, the dystocia was corrected by fetotomy and the animals were divided into two groups. Group I buffalo (n=20) were a) Oxytocin (50IU I/M) immediately and 4h after fetotomy, b) Prostaglandin $F_{2\alpha}$ (500µg I/M on d7 and d21 postpartum), and c) GnRH (buserlin acetate @ 20µg I/M) on day 28 postpartum. Group II buffalo (n=20) were injected only with routine antibiotics, analgesics and calcium. Group III comprised of 10 normally calved buffaloes and were not given any medication.

All the buffaloes subjected to fetotomy were sampled for blood and uterine fluid immediately after obstetrical treatment and subsequently on d7, 14, 21 and 28 postpartum. An autoclaved one-way stainless-steel catheter 15 inches length and 3 mm diameter was used for collection of uterine fluid with the help of a sterile syringe. Five ml of the aspirated fluid was transferred to a sterile plastic tube, placed in ice, and transported to laboratory within 4 h of collection for further analysis. A drop of the uterine fluid collected was put on a clean grease free slide and a smear was prepared immediately. The smears were air dried, wrapped in a tissue paper and transported to laboratory. The smear was stained with Leishman stain for 10 min and allowed to dry on slide warming stand. Under a microscope (100x), 100 cells per slide were calculated for total percentage of PMN cells and lymphocytes.

The uterine dynamics were obtained by palpation

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per rectum during postpartum period. The complete involution was judged on rectal palpation by return of uterus to its normal location in the pelvic cavity, normal and approximately equal size of uterine horns, and attainment of normal uterine texture, tone and consistency. On each per rectal examination, the observations regarding location and size of uterine horns and cervix, consistency and tonicity of uterine musculature, degree of reduction in size of gravid and nongravid uterine horns, exhibition of ovarian activity were recorded. In addition, exhibition of behavioural estrus signs and service period postpartum were also recorded.

The data was analysed by SPSS software programme and one-way analysis of variance. The significant interactions were tested using Duncan's multiple range test.

RESULTS AND DISCUSSION

On d7 postpartum, in all the buffaloes, uterus and cervix were hanging below pelvic symphysis and located in the abdominal cavity. By d14, 80% and 100% buffalo of group I and III had their genitilia returned to pelvic brim and pelvic cavity, respectively, whereas, the group II buffalo had their genitilia still in abdominal cavity. On d28 postpartum, all the animals in group I and III had their genitilia in pelvic cavity, whereas in group II only 60% had their genitilia in pelvic cavity. The diameter of cervix, non-gravid and gravid uterine horn were 4.70±1.00, 4.20±0.74, >5.0 cm and 7.00±2.91, 5.50±1.22, >5.0 cm in group I and II, respectively as compared to 3.50±0.70, 3.16±3.36 and 3.20±2.83 cm, respectively in control group on d28 postpartum.

Polymorphonuclear (PMN) cells in the uterine fluid of buffaloes of group I and II were higher ($p<0.05$) than group III during postpartum period (Table 1). However, PMN cells in group I buffaloes on d28 postpartum were lower ($p<0.05$) than group II buffaloes (Table 1), which indicated better uterine environment in group I.

Table 1: Polymorphonuclear (PMN) cells in uterine fluid as well as postpartum (pp) reproductive behavior of fetotomy operated and control buffaloes

Parameter	Day	Ecbolic+GnRH, n=20	No Ecbolic/ GnRH, n=20	Control, n=10
PMNs	7	68.10 ± 2.16^{C2}	83.10 ± 1.56^{C3}	60.80 ± 3.00^{D1}
	14	66.50 ± 4.22^{BC2}	75.10 ± 2.95^{B3}	50.20 ± 2.57^{C1}
	21	60.80 ± 1.27^{B2}	72.30 ± 4.09^{B3}	46.00 ± 4.33^{B1}
	28	56.85 ± 5.90^{A2}	63.10 ± 2.39^{A3}	41.60 ± 0.74^{A1}
First postpartum behavioural estrus				
Days		110.2 ± 6.2^{D}	127.4 ± 6.4^{E}	65.00 ± 3.53^{F}
Range, days		$80\text{-}128^{2}$	$86\text{-}135^{23}$	$55\text{-}75^{1}$
Estrus in d<120 pp		$95\%^{3}$	$30\%^{2}$	$100\%^{1}$
Estrus in d>120 pp		$5\%^{1}$	$70\%^{2}$	-
Postpartum fertility				
Service period, days		111.20 ± 6.15^{D}	127.60 ± 6.38^{E}	88.60 ± 4.80^{F}
Range, days		$105\text{-}148^{2}$	$116\text{-}156^{3}$	$74\text{-}122^{1}$
d75-120 pp		$30\%^{2}$	-	$90\%^{1}$
d≥120 pp		$70\%^{1}$	$30\%^{2}$	$10\%^{3}$
Conception rate				
d120 pp		70%	30%	80%

$^{A,B,C}p<0.05$, within the groups; $^{D,E,F,1,2,3}p<0.05$, between the groups

During postpartum period, the number of days for the occurrence of first behavioural estrus, animals showing estrus within d120 postpartum, overall service period, animals subjected to AI within study period as well as conception rate were better in group I (ecbolic+GnRH) compared to group II (no ecbolic or GnRH; Table 1).

The bacterial presence in uterus is usual in >90% of cows in first 10-14 days postpartum, regardless of disease signs (Sheldon and Dobson, 2004). In the present study, the average bacterial count (CFU/ml) in buffaloes subjected to fetotomy was higher ($p<0.05$) than the eutocic buffaloes on all days of sampling, which increased from d7 postpartum to d14 postpartum, followed by a decline in all the groups. On d28 postpartum, average colony counts were 240.00±45 CFU/ml, 354±66 CFU/ml and 82.00±9 CFU/ml in group I, II and III, respectively.

Thus, the administration of $PGF_{2\alpha}$ in early puerperum had a positive chronotropic effect on the uterine musculature, which facilitates quick expulsion of lochia and induction of estrus. The latter is responsible for physical expulsion of bacterial contaminants and inflammatory products as well as a possible improvement in uterine defences under low progesterone (Kasimanickam *et al.,* 2004).

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EFFICACY OF NEEM SEED EXTRACT±ASHWAGANDHA FOR THE TREATMENT OF ENDOMETRITIS IN BUFFALOES

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ABSTRACT

Repeat breeder buffaloes (n=36) with subclinical uterine infection tested positive by pH evaluation and White side test of cervico-vaginal mucus (CVM) and endometrial cytology were divided equally into three groups. Group-I containing fresh endometritic buffaloes (repeated ≤7 times) was administered once with Methanolic Neem seed extract (100 mcg/ml) @ 30 ml by intrauterine (IU) route, Group-II consisting of chronic endometritic buffaloes (repeated >7 times) was administered with Methanolic Neem seed extract (100 mcg/ml) @ 30 ml IU once only plus 15 gm Ashwagandha powder by oral route for 5 days and Group-III as a control group was administered with placebo as 30 ml PBS through IU. In both treated groups, pH of CVM deceased ($p<0.05$) after treatment and white side test turned negative in all recovered buffaloes. Polymorphonuclear cell count was reduced ($p<0.05$) post-treatment in recovered cases. Immunoglobulin level in serum of chronic endometritic buffaloes increased ($p<0.05$) post-treatment. A higher recovery rate was observed in Group-II as 75% against 66.7% in Group-I and nil recovery in control group. Pregnancy rates were recorded as 62.5%, 55.5% and nil in group-I, II and III, respectively.

Keywords: Ashwagandha, Buffaloes, Efficacy, Endometritis, Methanolic Neem seed extract

INTRODUCTION

Antimicrobial drugs hamper the uterine defense mechanism by inhibiting the phagocytic activity of polymorphonuclear leucocytes (PMNs). Besides, antimicrobial drugs have many disadvantages like high cost of treatment, reduced milk yield, development of resistance and varying success rate (Shukla and Pandit, 1989). Burgeoning literature cite the antimicrobial potential of many herbs like Neem, Garlic, Ginger, Tulsi, Turmeric and Aloe vera. Neem (*Azadirachta indica*) was extensively used in India as traditional Ayurvedic medicine for the treatment of various diseases. It was demonstrated that Neem has immuno-modulatory, anti-inflammatory, antifungal, antibacterial, antiviral, and antioxidant properties (Kumar, 2014). Recent studies have proved the efficacy of aqueous Neem seed extract to cure endometritis in both cows and buffaloes (Thombre, 2017).

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MATERIALS AND METHODS

Repeat breeder buffaloes were examined transrectally for the confirmation of normal genitalia. The buffaloes having poor body condition score and pathology of genitalia like cervicitis, kinked cervix and ovaro-bursal adhesions were excluded. The buffaloes with alkaline pH (>7.5) of CVM, positive white side test and increased PMN cell count (>4%) in endometrial cytology were considered as positive for endometritis. Total 36 buffaloes were selected for present research trial. On the basis of insemination record from owners, the cases were classified into fresh and chronic endometritic. The cases with history of repeat subsequent inseminations at seven times or less were considered as fresh endometritic cases and more than seven times were considered as chronic endometritic cases.

The CVM samples were collected in petridish with help of 20 ml syringe and AI sheath on the day of oestrus (pre and post treatment) for pH estimation by digital pH

meter and White side test. The positive and negative CVM samples by White side test were scored as '1' and '0', respectively. Blood samples were collected and serum was separated for estimation of general serum immunoglobulin level. General immunoglobulin levels were estimated using immuno-flocculation test (Deshpande *et al*., 1991) which is quantitative and rapid test. Endometrial smear prepared by cytobrush technique and smear was prepared and stained with Geimsa stain.

The selected buffaloes with endometritis were equally divided into three groups *viz*. Group-I consisting of fresh endometritic buffaloes (n=12) were treated with100 mcg/ml Methanolic Neem seed extract @ 30 ml by IU route once on the day of estrus; Group-II comprising of chronic endometritic buffaloes (n=12) were at par with Group-I plus oral Ashwagandha powder 15 gm in feed for 5 days. Group-III buffaloes as control were administered with placebo treatment as 30 ml PBS solution by IU route on the day of oestrus.

Data pertaining to pH, PMN cell count, recovery and pregnancy rate was analyzed statistically by using complete randomized design and ANNOVA.

RESULTS AND DISCUSSION

Buffaloes were diagnosed for endometritis on the basis of alkaline pH of CVM, positive White side test and PMN cell count >4% (Gilbert *et al.,* 2005; Barlund *et al*., 2007; Singh *et al.,* 2016) and selected for the experiment to evaluate comparative efficacy of two herbal protocols. White side test was efficitively used previously also for the diagnosis of endometritis in buffaloes (Puro, 2016; Thombre, 2017).

Mean pH of CVM in all the selected buffaloes was 8.5±0.1 before treatment (Table 1). The current observation regarding pH of CVM in repeat breeder buffaloes corroborates with earlier findings (Kumar *et al*., 2004). After treatment, average pH, white side test and PMN cell count differed ($p<0.05$) in recovered animals from both treatment groups. However, the same in control group as well as non-recovered buffaloes was similar ($p>0.05$, Table 1). The immunoglobulin flocculation test in serum yielded mean immunoglobulin level as 10.3 mg/ml in group-II and control buffaloes before treatment and that same increased ($p<0.05$) in Group-II buffaloes.

The buffaloes with fresh endometritis recovered after intrauterine infusion of methanolic Neem seed extract with a recovery rate of 66.7% in Group-I. Higher recovery rates as 75-80% were reported in previous studies for the treatment of endometritis in buffaloes using Neem seed oil through IU route (Kumar *et al*., 2009, 2013c). However, 87.5% and 75% recovery rates were documented earlier after treatment with Methanolic and acetonic Neem bark extract, respectively, in endometritic cows (Kumar *et al*., 2013b). Further, in the current study, the mean pH value reduced ($p<0.05$) from 8.6±0.1 to 7.5±0.1 in recovered animals while, the same was similar ($p>0.05$) in non-recovered and control buffaloes (Table 1). The mean PMN cell count in endometrial cytology reduced ($p<0.05$) from 14.8±1.9% to 2.5±0.6% in Group-I, however, it remained almost similar in non-recovered and control cases ($p>0.05$, Table 1).

The combined herbal therapy in Group-II buffaloes with chronic endometritis yielded 75% recovery rate as against nil recovery in control group. No report of similar herbal combination for the treatment of endometritic buffaloes and even cows could be traced from published literature for the comparison of current findings. However, the efficacy of herbal blends likes oral Ashwagandha powder with IU Garlic extract (Rahi *et al*., 2013) and IU Ashwagandha extract with oral Gilloy extract (Kumar *et al.,* 2017) in cows was studied with 75% recovery rate. The mean pH value in this group declined ($p<0.05$) from 8.5±0.1 to 7.5±0.01 after treatment and the same was similar ($p>0.05$) in non-recovered and control buffaloes. Further, the average PMN cell count reduced ($p<0.05$) from 13.7±2.1% to 2.3±0.6% after treatment in recovered cases, whereas it remained almost unchanged in non-recovered and control cases (Table 1). The mean

Table 1: Mean pH and PMN cell count before and after treatment in endometritic buffaloes

Treatment	Before treatment		After treatment			
			Recovered		Non-recovered	
	pH	PMN, %	pH	PMN, %	pH	PMN, %
Neem extract	8.6±0.1[a]	14.8±1.9[a]	7.5±0.1[b]	2.5±0.6[b]	8.5±0.1	14.0±2.9
Neem + Ashwagandha	8.5±0.1[a]	13.7±2.1[a]	7.5±0.1[b]	2.3±0.6[b]	8.4±0.3	12.0±3.1
Control	8.1±0.1	14.5±1.5	-	-	8.3±0.1	13.7±1.6

[a, b]$p<0.05$

serum immunoglobulin level increased ($p<0.05$) in recovered cases from 10.5±0.9 to 15.3±0.7 mg/ml after treatment, whereas, it declined slightly ($p>0.05$) in non-recovered cases (9.1±0.1 mg/ml) and in control group (9.5±.07 mg/ml).

Pregnancy rate in Group-I and II was 62.5 and 55.5%, respectively as against nil pregnancy in control group. The pregnancy rate in present study was higher than earlier studies who recorded only 25% pregnancy rate in endometritic repeat breeder cows treated with Methanolic Neem seed extract (Kumar *et al*., 2013a), whereas, the same was reported by as 44.4% in endometritic buffaloes treated with aqueous Neem seed extract (Thombre, 2017).

Thus, the combination therapy (Group-II) yielded higher recovery rate (75%) as against with bare Neem seed extract (Goup-I, 66.7% recovery). Conversely, the pregnancy rate was recorded higher in Group-I (62.5%) as against Group-II (55.5%).

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HIGH BLOOD / MILK UREA NITROGEN HAD DELETERIOUS IMPACT ON FERTILITY PARAMETERS IN CROSSBRED COWS

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ABSTRACT

Eighty-two healthy and recently calved crossbred cows from seven dairy farms were subjected to monthly blood and milk sampling for a four-month period for blood and milk urea nitrogen (BUN/MUN) assessment. The fertility parameters like calving to first estrus interval, calving to first service interval, calving to conception interval, services per pregnancy and percent animal conceived were recorded. The cows with high BUN or MUN had similar number of days to exhibit estrus and to first service compared to their counterparts ($p>0.05$). The cows with low BUN or MUN had lesser number of days to conceive than those with high BUN ($p<0.05$) or MUN ($p>0.05$). The percent animals conceived followed a similar trend in both BUN and MUN groups; low level groups had higher ($p<0.05$) conception rates. The number of services per conception was less among cows having low BUN or MUN. In brief, high BUN/MUN have deleterious effects on fertility of lactating crossbred cows.

Keywords: BUN, Crossbred cows, Fertility, MUN, Protein

An improvement in milk production in a crossbred cow warrants feeding strategies like feeding of high protein diet, however, this leads to an increase in blood (BUN) or milk (MUN) urea nitrogen. The monitoring of BUN or MUN is a reliable technique that can be used for measuring protein status of cattle. However, it is not known how the levels of urea in circulation, consequent upon feeding of protein rich diet would affect the reproductive efficiency of crossbred cows.

Recently calved apparently healthy crossbred cows (n=82) were selected from seven organized private dairy farms. Each animal was subjected to four blood and milk samplings on a particular date of each month starting from first month of their calving (first sample taken within 5-30 day after calving). Both blood and milk samples were analyzed for Blood Urea Nitrogen (BUN) and Milk Urea Nitrogen (MUN), respectively using commercial kits (BUN - Ortho-Clinical Diagnostics India Pvt. Ltd., Thane; MUN - Bayer Diagnostics, India) under recommended protocols. To calculate BUN and MUN of individual postpartum animals; Firstly, mean BUN/MUN values of all 4 months was calculated for each individual animal and then their overall means were calculated for all animals. The animals with values above and equal to mean BUN (15.03 mg/dl) and MUN (13.07 mg/dl) were considered under high BUN/MUN groups and the others with values below mean BUN/MUN were considered under low BUN/MUN groups.

The fertility parameters of each cow like calving to first estrus interval, calving to first service interval, calving to conception interval, service per conception and percent animal conceived were recorded from the respective farms. The data were analyzed statistically using SAS 9.2 software. Significance level of percentage animals conceived between the groups was calculated using CHI Square Test. Significance level of fertility parameters was calculated using Unpaired T Test.

The cows with high BUN or MUN had similar number of days to exhibit estrus and to first service post calving as their counterparts with low BUN or

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Table 1: The comparison of fertility parameters between High BUN / MUN or Low BUN / MUN exhibiting crossbred cows.

Parameter(s)	Blood urea nitrogen (BUN)		Milk urea nitrogen (MUN)	
	High BUN, >15.3 mg/dl, n=46	**Low BUN, <15.3 mg/dl, n=36**	**High MUN, >13.1 mg/dl, n=37**	**Low MUN, <13.1 mg/dl, n=45**
Calving-1st estrous interval, d	89.5±1.9	84.4±2.4	89.1±2.4	85.3±1.9
Calving-1st service interval, d	93.9±2.2	90.7±2.6	93.2±2.8	91.8±2.0
Calving-conception interval, d	121.6±5.6	104.0±4.7*	113.0±4.2	108.6±8.4
% conceived	45.6	75.0*	32.4	80*
Services/conception	2.1±0.1	1.7±0.1	2.1±0.1	1.8±0.1

*p<0.05; between groups for BUN or MUN

MUN (p>0.05, Table 1). Similarly, high BUN (Harris, 1992) or MUN (Larson *et al.,* 1997) had no impact on days to first estrus and days to first service intervals. Others observed that cows with low MUN (<10 mg/dl) presented longer intervals between calving to first estrous and calving to first service with justification that animals fed with low protein resulted in delayed post-partum ovarian activity (Carlsson and Pehson, 1993). Furthermore, cows with high MUN also exhibited significant extension in calving to first estrous interval (Veena *et al*., 2016).

The cows with low BUN or MUN took lesser number of days to conceive than those with high BUN (p<0.05) or MUN (p>0.05, Table 1) as reprted earlier (Rajala-Schultz *et al.,* 2001). The percent cows conceived followed a similar trend in both BUN and MUN groups, and low-level groups had higher (p<0.05) conception rates (Table 1), as reported earlier (Tshuma *et al*., 2014).

The logic for lower fertility in cows having high BUN has been attributed to uterine pH, which decreased approximately by 0.1 pH units for each 5 mg/100 ml increase in BUN. These changes might have affected the uterine environment, which in turn retarded the embryo development and hence, influenced conception adversely. Alternatively, the reason behind negative association between high BUN and reproductive performance could be due to the concurrent energy deficit in such animals (Gulinski *et al.,* 2016). Most of studies pointed out that BUN/MUN at >19 mg/dl and <7 mg/dl could be detrimental to fertility. Since the cut off level in the present study was 15.5 mg/dl for BUN and 13.07 mg/dl for MUN, therefore, it could be inferred that fertility was not affected to that extent as at the cut-off level of 19 mg/dl.

To conclude, high BUN/MUN could have deleterious effects on fertility of lactating crossbred cows. Therefore, the periodic screening for BUN/MUN during postpartum period should be encouraged that would provide early insights regarding proactive dietary protein adjustments for achieving optimum reproductive efficiency.

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CERVICAL MUCUS FERN PATTERN IN RELATION TO FERTILITY AND MICRO-MINERAL PROFILE IN ANESTRUS SURTI BUFFALOES SUBJECTED TO OVSYNCH ALONE AND IN COMBINATION WITH PRID

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ABSTRACT

Eighteen anestrus Surti buffaloes were subjected to Ovsynch or Ovsynch plus PRID or inseminated at spontaneous estrus (n=6 in each group). Overall conception rate was 77.8% in buffaloes with typical fern pattern as compared to 42.8% with atypical fern pattern. Serum copper, zinc, iron and manganese concentrations were similar (p>0.05) between animals during the study period.

Key words: Anestrus, Buffalo, Cervical mucus, Fern pattern, Ovsynch, Surti

Low reproductive efficiency in buffaloes remains a major economic problem globally. Various hormonal preparations were used in anestrus and subestrus buffaloes with variable success (Rathore *et al.,* 2006; Singh *et al*., 2006). The present study investigated the impact of cervical mucus fern pattern and serum micro-mineral profile on conception rate in anestrus Surti buffalo subjected to estrus synchronization.

Eighteen Surti buffaloes between 45 and 120 days postpartum were included in the present study. Estrus was detected daily with the help of teaser bull parading during morning and evening hours. The animals not exhibiting overt signs of estrus were segregated and subjected to rectal palpation. The animals with smooth ovaries on rectal palpation at eleven-day interval were divided at random into three groups of six animals each. The first group was subjected to standard ovsynch protocol followed by fixed time inseminations (FTAI) twice, morning and evening, on day 10 of protocol. The second group buffaloes were inserted PRID (0.9 g of progesterone) intra-vaginally which was kept *in-situ* from day 0 to 7 of ovsynch protocol. Rest of the ovsynch protocol and FTAI was same as in group-1. The third group control (no hormone therapy) buffaloes were inseminated at spontaneous estrus. The pregnancy was confirmed per rectally 60 days of last AI.

The procedures for arborization or fern pattern of cervico-vaginal mucus collected befrore AI were carried out as per the known standard pattern using a microslide. Blood samples (5-6 ml) were collected from all animals on day 0 (prior to treatment), day 4 (during treatment), day 8 (after cloprostenol inj.), and day of estrus / FTAI and on day 28 (18^{th} day post-AI) by jugular vein puncture in serum clotting vaccutainers, and serum separated was stored at –20°C until analysis. Serum was subjected to estimation of trace elements (copper, zinc, iron and manganese) on an Atomic Absorption Spectrophotometer.

The test of significance among and within the groups for micro elements profile was made by analysis of variance and the mean differences between and within the groups were tested using Duncan's multiple range test at 5% level of significance.

In the present study, the overall conception rate, irrespective of estrus synchronization group, was higher (77.8 *vs.* 42.8%, p>0.05) in buffaloes with

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typical compared to atypical fern pattern. However, the serum copper, zinc, iron and manganese and in acyclic Surti buffaloes was similar ($p>0.05$) within as well as between all the treatment and control groups including overall mean values at different days.

In brief, similar concentrations of various serum micro-minerals values between treated and control groups at different days or between groups suggested optimum nutritional supplementation and healthcare strategies adopted in the organized farm of Surti buffaloes. Nevertheless, the typical fern pattern was associated with better chances of conception in Surti buffalo.

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ROLE OF EFFECTIVE COMMUNICATION IN ENHANCING DOG OWNER KNOWLEDGE ABOUT BREEDING PRACTICES IN PUNJAB

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ABSTRACT

At Teaching Veterinary University Hospital and various districts of Punjab, the dog owners (100 each from urban and rural area) were randomly selected and personally interviewed using pretested interview schedule. Breeding knowledge score of both groups was different ($p<0.05$), however, the knowledge level of both groups was under low category. A positive correlation ($p<0.05$) existed between breeding practice knowledge index and their communication profiles. The present study underlines the importance of communication profile in improving the knowledge level of dog owners about correct breeding practices.

Key words: Breeding practice, Communication, Dog, Knowledge, Punjab

Breeding, an essential component in canine rearing, is an art as well as science as it needs skill and knowledge. A responsible breeder should take care of dog before pregnancy, during pregnancy and after whelping (Ivanova and Georgiev, 2018). Based on preventive measures, proper managemental practices and knowledge level of dog owner, the outcome of pregnancy depends. The extension machinery serves as a useful media for the dissemination of latest technological knowledge to dog owners via various communication methods. The present study was carried out to assess the knowledge level of dog owners about breeding practices and correlation of these breeding knowledge levels with communication profiles like extension contact, social participation and mass media exposure.

The present study was conducted in Teaching Veterinary Clinical Complex (TVCC) of the University and in different districts of Punjab state. The randomly selected dog owners (n=200) were categorized in Group I (Urban dog owners) and Group II (Rural dog owners). The data was collected through pretested interview schedule by personally interviewing the dog owners. For breeding practice, dog owners were categorised into 3 categories based on the knowledge scores as low, medium and high knowledge with scores 0-≤8, >8-16, and >16 respectively. The knowledge index was calculated by dividing obtained knowledge score by maximum possible score and multiplying the result with 100.

The extension contacts of dog owners with veterinary officers/ university/ breeders were measured on two-point continuum either as 'No' (score 0) or 'Yes' (score 1). Hence, the maximum score for extension contact could be 6. The social participations of dog owners in dog shows/ Livestock championship/ Pashu Palan or Kisan Melas and Animal welfare camps were measured on two-point continuum either as 'No' (score 0) or 'Yes' (score 1). Hence, the maximum score for social participation could be 8. The mass media exposure of dog owners with television/ radio/ newspaper/ dog magazines/ internet/ mobile phone/ books were measured on two-point continuum either as 'No' (score 0) or 'Yes' (score 1). Hence, the maximum score for mass media exposure could be 14. The collected date was tabulated and analysed with the help of SAS 9.3 system Carry N C, USA.

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Table 1: Spearman Correlation coefficient of breeding knowledge index of all dog owners with communication profile.

Spearman correlation coefficient, n=200	Breeding practice knowledge index (BPKI)	Extension contact (EC)	Social participation (SP)	Mass media exposure (MME)
BPKI	1	0.65748*	0.54317*	0.66914*
EC	0.65748*	1	0.56284*	0.31778*
SP	0.54317*	0.56284*	1	0.42269*
MME	0.66914*	0.31778*	0.42269*	1

*p<0.05

According to communication profile, 50% of urban dog owners and 70-76% of rural dog owners had low extension contact and social participation. Overall, a very few dog owners had high extension contact (10%), social participation (6%) and mass media exposure (22.5%).

Regarding dog breeding practices, 26.4% urban and 14.6% rural dog owners had awareness. In fact, 70% urban and 95% rural dog owners were not aware about breed specific characters of dogs. Only 5% rural dog owners have knowledge about number of times a male dog used for service in a week and knowledge about the day of mating. Very few dog owners had knowledge about vaginal cytology, care during pregnancy, complications during pregnancy and whelping, pseudo pregnancy, sexually transmissible diseases, venereal granuloma, causes of pregnancy loss and new borne pup care. Others suggested that owner knowledge about length of gestation, methods for pregnancy diagnosis, weight management, parturition signs and timings are more important (Fontaine *et al*., 2007).

The breeding knowledge score was high (6.34±0.37) in urban compared to rural (3.51±0.47, p<0.05) dog owners, however both groups had low knowledge score. Furthermore, there was a positive (p<0.05) correlation of breeding knowledge index of dog owners with extension contact, social participation and mass media exposure (Table 1). Others reported that extension contact, exposure to extension mass-media, management orientation and innovation proneness among dog owners of 3 urban cities of Gujarat state had significant relationship with knowledge of dog owners (Raval *et al.,* 2015). This shows that communication profile plays an important role for improving knowledge level of dog owners relating to dog breeding practices.

In brief, the present study highlights the difference in knowledge level of urban and rural dog owners abut breeding practices, thus, suggesting the need of organising extension programmes for enhancing their knowledge. Also, the communication profile of dog owner was correlated (p<0.05) with knowledge level. This indicates that participation of owner in extension drive can enrich their knowledge level.

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INFLUENCE OF TYPE OF WHELPING ON NEONATAL MORTALITY IN PUPS

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ABSTRACT

The puppies (n=100) born to bitches that underwent Spontaneous Whelping (SW), Assisted Whelping (AW) and Caesarean section (CS) were divided into groups as SW (n=30), AW (n=35) and CS (n=35). Out of 100 puppies, 25% were born dead (2% SW, 7% AW and 16% CS). Among viable pups at birth, the early neonatal mortality till 24 h was 18% (0% SW, 5.7% AW and 45.7% CS). In brief, the stillbirth and early neonatal mortality was high in pups delivered through caesarean compared to spontaneous or assisted whelping.

Key words: Assisted whelping, Caesarean section, Neonatal mortality, Pups, Spontaneous whelping

The newborn puppy is an immature animal, dependent on its dam for survival in the first three weeks and as a consequence the etiology of neonatal death is frequently complex and often undetermined (Blunden, 1998). The majority of pup losses are stillbirths and deaths within the first week of life known as perinatal mortality. The mortality can occur *in utero*, during expulsion, after birth, in first weeks of life or after weaning. The causes for high pup mortality in perinatal period can relate to several factors concerning bitch (mismothering, lack of milk, trauma), birth process (prolonged labor, dystocia, obstetrics), puppy (low birth weight, congenital malformations, starvation), environment and presence of infectious agents (Munnich, 2008).

The puppies born to bitches (age, 2-6 yr) of different breeds with the history of progressive whelping and / or dystocia were used in the present analysis. A total of 100 puppies born to bitches that underwent Spontaneous Whelping (SW), Assisted Whelping (AW) and Caesarean section (CS) were selected. The puppies born to five bitches without any medical, manual or surgical assistance either to dam or puppies were kept in group I (SW, n=30).

The puppies born to ten bitches that delivered through either manual or medical assistance were kept in group II (AW). Manual assistance was attempted to puppies which were partly expelled from the vagina and/or the puppies whose parts were within reach on vaginal examination but not progressed further. The bitches were allowed in standing position or restrained on their lateral recumbency and the vaginal passage was well lubricated with liquid paraffin. The body of puppies were grasped gently and pulled steadily away from the bitch caudo-ventrally. While applying traction, care was taken to ensure that force was not applied on the limbs of puppies. Induction protocol was initiated with slow intravenous injection of oxytocin @ 1.1-2.2 IU/kg IM or SC with a dose range between 5-20 IU every 30 min and concurrent administration of 10% calcium gluconate @ 0.5-1.5 ml/kg to augment the effect of oxytocin on myometrial contraction and intravenous fluids to correct hydration, electrolyte, and blood glucose abnormalities. In between, whenever the bitches were straining, manual assistance was given to deliver the puppies.

The puppies born to nine bitches that underwent caesarean section were in group III (CS). Under general anaesthesia using propofol @ 3 mg/kg and diazepam @ 0.5mg/kg for induction and 2% isoflurane for maintenance, caesarean section was performed

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through mid-ventral approach by adopting standard surgical procedures.

Out of 100 pups evaluated, 25% were born dead (6.7% SW, 20% AW, 16% CS), 8% died within 30 min (0% SW, 5.7% AW, 17.1% CS), 6% died within 2h (0% SW and AW, 17.1% CS), 4% died in 2-24h (0% SW and AW, 11.4% CS), and 57% survived beyond 24h (93.3% SW, 74.3% AW, 8.6% CS).

The incidence of born dead or stillbirth in the present study (25%) is higher than earlier studies reporting between 4.3-11.5% (Indrebo *et al*., 2007; Tonnessen *et al*., 2012), that could be due to higher stillbirths in CS group which were delayed cases. Further, the effect of fetal distress arising from dystocia or the fetus retained for long time in the uterus or birth canal or exposed to effects of oxytocin prior to resorting emergency CS may be responsible for increased incidence of stillbirths in present study (Gropetti *et al*., 2010; Jayakumar *et al*., 2015). Hence, most of the still born puppies may be saved if timely adequate veterinary assistance is given.

Among viable pups at birth, the early neonatal mortality till 24 h was 18% (0% SW, 5.7% AW and 45.7% CS) in the present study, which was higher when compared to an earlier report (Potkay and Bacher, 1977). The high incidence of neonatal mortality was mainly in pups delivered through CS which might be due to hypoxia, hypoglycemia and hypothermia due to delayed whelping (Münnich and Küchenmeister, 2014) followed by the effects of anaesthetic agents on the cardiac, respiratory and nervous systems during CS. Hence, the number of early neonatal mortalities may be reduced if CS is performed at the earliest.

In brief, an early and adequate veterinary assistance can reduce the incidence of stillbirth and neonatal mortality in pups.

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SERUM METABOLIC AND MACRO-MINERALS PROFILE IN POSTPARTUM ANESTRUS SURTI BUFFALOES TREATED WITH OVSYNCH ALONE AND IN COMBINATION WITH PRID

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ABSTRACT

Eighteen anestrus Surti buffaloes were subjected to Ovsynch or Ovsynch plus PRID or inseminated at spontaneous estrus (n=6 in each group). Serum protein and cholesterol was higher ($p<0.05$) in synchronized buffaloes compared to their control counterparts. Serum glucose, calcium, phosphorus and magnesium concentrations were similar ($p>0.05$) between animals during the study period.

Key words: Anestrus, Buffalo, Metabolic, Mineral, Ovsynch

True anestrus is a result of suppression of reproductive hormone release through the effect of lactation, nutrition and systemic diseases. This study assessed the metabolic and macro-mineral profile in postpartum Surti buffaloes subjected to estrus synchronization protocol.

Eighteen Surti buffaloes between 45 and 120 days postpartum were included in the present study. Estrus was detected daily with the help of teaser bull parading during morning and evening hours. The animals not exhibiting overt signs of estrus were segregated and subjected to rectal palpation. The animals with smooth ovaries on rectal palpation at eleven-day interval were divided at random into three groups of six animals each. The first group was subjected to standard ovsynch protocol followed by fixed time inseminations (FTAI) twice, morning and evening, on day 10 of protocol. The second group buffaloes were inserted PRID (0.9 g of progesterone) intra-vaginally which was kept *in-situ* from day 0 to 7 of ovsynch protocol. Rest of the ovsynch protocol and FTAI was same as in group-1. The third group control (no hormone therapy) buffaloes were inseminated at spontaneous estrus. The pregnancy was confirmed per rectally 60 days of last AI.

Blood samples (5-6 ml) were collected from all animals on day 0 (prior to treatment), day 4 (during treatment), day 8 (after cloprostenol inj.), and day of estrus / FTAI and on day 28 (18^{th} day post-AI) by jugular vein puncture in serum clotting vaccutainers, and serum separated was stored at –20°C until analysis. Serum was subjected to estimation of serum metabolic profile (glucose, protein, cholesterol) as per standard procedures, as well as macro-mineral profile (calcium, phosphorus and magnesium) using an Atomic Absorption Spectrophotometer.

The test of significance among and within the groups for micro elements profile was made by analysis of variance and the mean differences between and within the groups were tested using Duncan's multiple range test at 5% level of significance.

Serum glucose in acyclic Surti buffaloes was similar ($p>0.05$) between days within and between treatment and control groups. (Table 1). However, serum total protein and cholesterol was higher in

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synchronized buffalo than control group. Similar values were obtained earlier in anestrus buffaloes (Kumar *et al.,* 2010; Parmar *et al*., 2015). Moreover, higher serum cholesterol concentration observed in Ovsynch plus PRID group compared to Ovsynch group was in agreement with earlier findings (Buhecha *et al.,* 2016). Serum macro-mineral profile (calcium, phosphorus and magnesium) of acyclic Surti buffaloes neither differed ($p>0.05$) within group nor between treatment and control groups at any of the intervals.

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SYNCHRONIZATION OF OVULATION USING DOUBLESYNCH PROTOCOL IN CROSSBRED CATTLE UNDER FIELD CONDITIONS

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ABSTRACT

The crossbred cattle (n=110) were randomly divided to receive either no treatment and were regular cycling (group 1, n=10), and the remaining (group II, n=100) were pubertal heifers, repeat breeders and anestrous. The animals were fed area specific mineral mixture @ 30 gm/d/animal for 45d and group II animals were subjected to doublesynch protocol followed by FTAI. Body condition scores (BCS) was accessed and ranged 2.4-3.4 in all the animals. Blood samples was collected for the estimation of plasma Beta hydroxy butyric acid (BHBA) and the animals that failed to conceive to doublesynch had higher plasma BHBA. The conception rate was 54% in synchronized compared to 40% in control group. Thus, doublesynch is a useful protocol to improve the conception rate in infertile cattle under field condition.

Keywords: BHBA, Conception, Crossbred cattle, Doublesynch, Hormone

Low fertility in cow has multifactorial etiology involving genetic improvement, inadequate nutrition and poor reproductive management. The partitioning of the relative impact of the various factors on infertility is not well understood. Doublesynch protocol has the potential to increase the pregnancy rates in primiparous dairy cows (Oztruk and Baran, 2009). Thus, the present study was conducted to assess the efficacy of doublesynch protocol in improving infertility in crossbred cattle under field conditions.

Healthy pluriparous crossbred cows and pubertal heifers free from any palpable abnormalities of the reproductive tract were randomly divided in two groups and provided with area specific mineral mixture for 45 days @ 30 gm/day. Group I (n=10) animals were regular cyclic with no failure of conception and these were inseminated at observed estrus, twice at 24h interval, without any treatment. The group II animals (n=100) consisted of repeat breeders (n=46) with no palpable abnormality of reproductive tract, anestrous animals (n=35) without corpus luteum in the ovary, and pubertal heifers (n=19) of >4 yr age with no reproductive tract abnormalities but failed to conceive. Group II was subjected to Doublesynch protocol ($PGF_{2\alpha}$ analogue @500 µg on day -2, followed by ovsynch protocol from day 0 onward) and subsequent FTAI as per standard procedures.

Body Condition Score (BCS) was assessed for all the animals as per the standard technique (Wildman *et al.*, 1982) and was recorded to be in the range of 2.4 to 3.4. Blood was collected at the time of screening, plasma was separated and stored at -20˚C for the estimation of BHBA by spectrophotometer. In all the groups, the intensity of estrus and duration of estrus were recorded and pregnancy was confirmed by rectal palpation on day 60 post AI. The accumulated data were analysed statistically.

Conception rate in group I and group II cows was 40% and 54%, respectively. Others also reported an increase pregnancy rate in cattle subjected to doublesynch protocol (Oztruk and Baran, 2009). Plasma BHBA levels in cattle that conceived or failed to conceive in group I was 0.45 mmol/l, whereas in group II, their values were 0.54 and 1.00 mmol/l,

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respectively. In fact, BHBA values >0.7 mmol/l indicate severe negative energy balance (Adewuyi *et al.,* 2004). In this study, even though doublesynch protocol was initiated to improve the conception rate, 46% of repeat breeders failed to conceive because of negative energy balance.

In present study, the conception rate in doublesynch treated repeat breeders as well as anestrous cattle was similar (56.5 *vs.* 54.3%, respectively), where as pleuriparous animals showed better conception rate than heifer (55 *vs.* 45%, respectively).

The intensity of estrus ranged from mild to intense, with animals showing moderate estrus in both the control (2/10) and treatment group (55/100) when compared with mild and intense estrus. The mean duration of estrum was 19.05, 18.75, 22.02 and 20.39 h in animals that conceived or failed to conceive in group I and group II, respectively.

It can be concluded from the present study that doublesynch protocol can be a useful tool to improve reproductive efficiency in crossbred cattle under field conditions.

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IMPROVEMENT IN CONCEPTION RATE BY GnRH OR hCG ADMINISTRATION ON DAY 5 POST-AI IN NON-INFECTIOUS REPEAT BREEDER COWS

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ABSTRACT

Thirty-six non-infectious repeat breeder cows were selected on the basis of typical fern pattern, negative white side test, normal pH of cervical mucus and <4% polymorphonuclear (PMN) cell count in endometrial content. Selected animals were randomly distributed in three groups containing twelve animals each. Group-I and Group-II were treated with inj. GnRH @ 10 mcg and inj. hCG @ 2000 IU by intramuscular route, respectively on day 5 post-AI, whereas group-III served as controls. Conception rates in group I, II and III was recorded as 58.3, 66.7 and 25.0%, respectively. Thus, hCG treatment on day 5 post-AI improved conception rate in non-infectious repeat breeder cows.

Key words: Conception rate, GnRH, hCG, Non-infectious, Repeat breeder

About 32-35% cases of repeat breeding in dairy animals are due to non-infectious causes and out of these early embryonic mortality is one of the most important cause (Diskin *et al*., 2008). Inadequate functioning of corpus luteum (CL) is responsible for early embryonic mortality in cattle (Kimura *et al*., 1987). Delayed leutinization may lead to inadequate release of progesterone during early embryonic period, thereby reducing the chances for embryonic survival. Several measures were adapted to correct luteal dysfunction including use of GnRH (Lewis *et al*., 1990) or hCG (Santos *et al.,* 2001) after insemination which increases progesterone secretion and renders high conception rate. Considering the post-fertilization reproductive events and endocrine changes, it is proposed to attempt GnRH and hCG therapies in non-infectious repeat breeder cows for improvement of conception rate.

Thorough clinical investigations were conducted to select the specific cases of repeat breeding due to luteal insufficiency. The cases were evaluated during estrus stage by per-rectal palpation as well as through laboratory investigations to rule out other etiological factors causing repeat breeding like improper timing of insemination or embryonic mortality due to uterine infection. The cervico-vaginal mucus (CVM) was collected in a sterilized petridish on the day of estrus simultaneously with per-rectal palpation. Fern patterns in cervico-vaginal mucus smear were evaluated under low power (10 X) and were classified as Typical type (fern pattern with primary, secondary and tertiary branches), Atypical type (fern pattern with primary and secondary branches) and Nil type having no fern pattern. Cervical discharges were also evaluated with digital pH meter. The observation of physical properties like color, consistency and odour of CVM reflected the presence of uterine infection and the same was confirmed with white side test (Bhat *et al*., 2014). Uterine mucus samples were aseptically collected by using cytobrush and smears were prepared and stained with Geimsa stain for PMN cell count under oil immersion using 1000X magnification. The positive cases for uterine infection as confirmed by white side

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test (yellow color), uterine cytology (PMN cells >4%) and pH (>7.5) were excluded and only non-infectious repeat breeder cows were selected for the study. Artificial insemination was performed only in the cows showing typical fern pattern. Thus, total 36 cases of repeat breeding cows were taken for the experiment in three groups *viz.* Group-I (n=12) cows were treated with inj. GnRH @ 10 mcg by intramuscular route and Group-II (n=12) cows were assigned with inj. hCG @ 2000 IU intramuscularly on day 5 post-AI whereas, Group-III (n=12) cows were kept untreated post-AI as a control. The effect of treatment in different groups was evaluated in terms of first service conception rate as confirmed by trans-rectal examination after 60 days of AI.

Typical type of fern pattern with tertiary branching in cervical mucus is indicative of ovulatory heat, whereas, atypical fern pattern is observed in silent or weak estrus (Galhotra *et al*., 1971). In present study, pH values of mucus sample in cows from group-I, II and III were recorded between 7.0-7.5. Samples of endometrial content were carrying <4% PMN cells with average number as 3.04±0.36% in group I, II and III. A positive correlation was reported between PMN cells and bacterial infection in uterus (Dutt *et al.*, 2017). If PMN cell count is >10%, the chances of subclinical endometritis are highly evident.

First service conception rate of group I, II and III was 58.3, 66.7 and 25.0%, whereas the number of services per conception was recorded as 1.7, 1.5 and 4.0, in the respective groups. The result of conception rate in group-I (58.7%) corroborated with an earlier study in GnRH treated non-infectious repeat breeder cows (62.5%; More *et al.,* 2012). In another study, higher conception rate (75%) was recorded (Pandey *et al.*, 2016) compared to current findings (66.7%) in repeat breeder crossbreed cows treated with hCG. Administration of hCG or GnRH in early luteal phase between d4-7 of estrus cycle induced pre-ovulatory luteinization of the dominant follicle of first wave and thereby formation of accessory CL. This may further aid in progesterone concentration with a positive impact on embryo survival (Mehni *et al*., 2012). Thus, the present study reflects that hCG treatment on day 5 post-AI was effective to achieve higher conception rate in non-infectious repeat breeder cows.

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IMPERFORATE HYMEN AND SUBSEQUENT MUCOVAGINA IN A FILLY

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ABSTRACT

An eighteen-month-old filly was presented with imperforate hymen and subsequent development of mucovagina. The hymen was incised and accumulated secretion was drained. The linear incision of hymenal membrane was bluntly extended through digital manipulation till smooth passage of hand in vaginal canal without any resistance and cervix was palpated. Animal recovered uneventfully, bred after seven months and diagnosed positive for pregnancy at three months of gestation.

Key words: Imperforate hymen, Mucovagina, Filly, Vaginoscopy

INTRODUCTION

In mare, congenital vaginal obstruction is most frequently associated to an imperforate hymen, an incomplete persistent hymen or a vaginal hypoplasia (Hughes, 1992; Freeman and England, 1997; Raggio *et al*., 2005). Generally, a variable degree of persistency of hymen is observed in maiden mare but in rare cases, an imperforate hymen is noted leading to accumulation of fluid within the vagina and uterus. Mostly, hymen is swept by fingers and hand before breeding a maiden mare but sometimes the hymen is so tough that it can only be ruptured using a guarded scalpel blade or scissors. The present case in a filly explains imperforate hymen with subsequent development of mucovagina and its surgical management.

CASE HISTORY AND OBSERVATIONS

A filly (age, 18 month) of upgraded Kathiawari breed had the history of straining while urination and defecation along with the frequent protrusion of a large part like a fluid filled balloon coming out of vulva (Fig. 1) when she lay down and also during canter. Filly was attended by local veterinary practitioner and suspected for vaginal prolapse and given treatment accordingly. But there was no relief over a week-long treatment thereafter case was referred to university hospital. Per rectal examination revealed a fluid filled fluctuating swelling in the vagina behind cervix, however, the uterine horns were normal on palpation. Transrectal ultrasonography revealed granular free-floating fluid distending the posterior vagina, however, a tough membrane was obstructing the hand to palpate cervix per vaginally. The vaginoscopic examination confirmed an imperforate membrane with similar appearance to the adjacent tissue but slightly less reddish and obstructing the visualisation of cervix (Fig. 2). The filly was diagnosed with imperforate hymen and subsequent development of mucovagina due to obstruction to natural drainage.

TREATMENT AND DISCUSSION

The filly was restrained properly in standing position under trevis and was administered epidural anaesthesia (2% lignocaine HCl; 8 ml in first coccygeal interspace). The tough hymenal membrane was taken out with the help of soft tissue holding forceps and an incision was made dorsal to ventral direction (Fig. 3) and around 2 L mucoid fluid without any off smell was drained off. (Fig. 4). The endoscopic examination revealed normal vagina and a tightly closed cervix (Fig. 5). The lubricated gloved hand was passed gently through vagina to tear hymenal membrane

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Fig. 1: Protrusion of Imperforate hymen

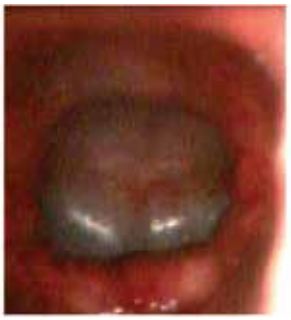

Fig. 2: Vaginoscopic appearance of persistent hymen

Fig. 3: Incision of hymen

Fig. 4: Flow of mucous after incision

to its maximum and to ensure smooth passage of hand. The vagina was flushed with normal saline 2-3 times and Xylocain jelly (4% lignocaine HCl) and Neosporin powder was smeared throughout vagina. The postoperative treatment of dam comprised mainly of injecting combination of antibiotic for five days. During the subsequent period, the filly had no vaginal discharge or associated systemic abnormalities. The tenesmus ceased and normal posture to urination and defecation was noted with no further protrusion of membrane from vulva. The filly was successfully breed after 7 months and three months after breeding, a positive pregnancy diagnosis was made by per rectal examination.

The hymen is formed from epithelial lining of paramesonephric ducts and urogenital sinus at the vestibulovaginal junction. The canalization of hymen is usually complete at birth and leads to communication between the lumen of caudal vagina and vestibule (Roberts, 1986). The most frequent developmental anomaly concerning the caudal reproductive tract in the mare is imperforate hymen or persistence of variable degree of hymen (Mc Entee, 1990; Hughes, 1992). In mare, few reports exist on developmental anomalies of cervix and cranial vagina besides those associated with pseudohermaphroditism and testicular feminization syndrome (Kieffer, 1976; Crabbe *et al*., 1992).

Hydrometra is a common sequale of vaginal obstruction in cyclic females as the normal outflow of the uterine secretions is prevented leading to accumulation of fluid with an increase in age and cyclic ovarian activity of the female (Troiano and McCarthy, 2004). Iin present case, filly was not reported for any cyclic symptoms though there was accumulation of vaginal secretion and epithelial debris. In this case, the cervix was closed as visualised by vaginoscope, thus, no chance of entry of vaginal secretion into uterus. The latter was confirmed by ultrasonography as uterine horns had no accumulation of secretions.

In cattle, the most common developmental aberration, due to a sex-linked recessive gene, of female reproductive tract is variable degree of hymen persistence with white shorthorn breed being most affected (Parkinson, 2001). The accumulation of secretions associated with complete hymen obstruction can be relieved by trocar and cannula. In present case, the obstruction was relieved by incision of hymenal membrane with scalpel and blade, thus leading to drainage of mucoid fluid. Nevertheless, the surgical intervention to enable successful breeding is not advisable due to hereditary origin (Parkinson, 2001). In horses and other ruminant species, persistent hymen is reported, but heritability is unknown.

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DYSTOCIA DUE TO BILATERAL HOCK FLEXION IN A JENNY (*EQUUS ASINUS*)

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ABSTRACT

A case of fetal dystocia due to bilateral hock flexion in a jenny and the successful *per-vaginum* delivery of fetus is reported through partial fetotomy using double barrel Thygesen fetotome.

Key words: Dystocia, Fetotomy, Hock flexion, Jenny

INTRODUCTION

The process of foaling in equines is a rapid (30 minutes) and violent process with very low incidence of dystocia (Bhoi *et al.*, 2010). Nevertheless, the majority of dystocia cases were of fetal origin with postural disposition being very common due to long foal extremities (Arthur *et al*., 1989). The present case report places on record the successful management of dystocia due to bilateral hock flexion in jenny through percutaneous fetotomy.

CASE HISTORY AND OBSERVATIONS

A four-year-old primiparous jenny had the history of unsuccessful labor since 24 h and the water bags had ruptured 18 h earlier. The case was handled by a local practitioner but failed to relieve the dystocia. The animal was dull, depressed and lying down with labored breathing and intermittent straining. The per vaginal examination revealed fully dilated and dry birth canal with variable degree of inflammation. Fetal tail was coming out from the vulva (Fig. 1), The fetus, whch was not emphysemated, was diagnosed in posterior presentation with bilateral hock flexion and fetal tail presented out of vulva (Fig. 1).

TREATMENT AND DISCUSSION

The birth canal was thoroughly lubricated with liquid paraffin, however, the attempts made to correct the hock flexion were unsuccessful. Hence, both the hind limbs were disarticulated at the level of hock using a double barrel Thygesen fetotome. After thorough lubrication of breech area of fetus and birth canal, initially one-point traction followed by simultaneous two points traction was applied just above the hock to ensure that both the stifle joints enter the pelvic cavity (Fig. 2). While applying traction, the precaution was taken to protect birth canal from being damaged by amputated stumps and a dead male fetus was extracted out. The jenny was administered with routine antibiotics and supportive therapy for four days along with local dressing of vaginal laceration. The animal recovered uneventfully as informed by owner.

Equine dystocia is a true emergency and threatens the survival of dam and fetus both (Freeman *et al*., 1999). Long extremities of foal tend to predispose a Jenny to dystocia (Chauhan *et al*., 2013), whereas, dystocia due to malformations like schistosomus reflexus and ankylosis of joints is also reported (Dubbin *et al.,* 1990). In the present case, partial fetotomy using double barrel Thygesen fetotome proved beneficial to deliver the malpostured foal *per-vaginum*.

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Fig. 1: Fetal tail presented at vulva

Fig. 2: Two-point traction on amputated hind limb

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SURGICAL MANAGEMENT OF TESTICULAR HYPERPLASIA IN A CRYPTORCHID LABRADOR RETRIEVER

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ABSTRACT

A 6-year-old Labrador retriever was presented with subcutaneous inguinal mass increasing in size. On scrotal palpation, the dog was bilaterally cryptorchid. Ultrasonography revealed mixed echogenic enlarged testicle, while the other could not be visualized. The surgical removal of subcutaneous testicular mass was done with uneventful recovery.

Key words: Cryptorchid, Dog, Surgical management

INTRODUCTION

Cryptorchidism occurs most commonly in stallion, boar and some breed of dogs like Boxers, Pomeranians, Dachshunds, Sealyhams, Cairn Terriers (Noakes, 2009). Among cryptorchid dogs, unilateral cryptorchidism is more common with 1-7% incidence (Noakes *et al.,* 2009 and Sridevi, 2015). This condition followed by development of neoplasia is common in stallions and dogs. Other complication in response to raised intra-testicular temperature and endocrine disturbance is testicular degeneration (Noakes *et al.,* 2009). In dogs, the etiology of canine cryptorchidism may be heritable and linked to sex-limited autosomal recessive trait. Although, non-genetic factors such as relative size of testis and inguinal canal may also be involved (Sridevi, 2015).

CASE HISTORY AND OBSERVATIONS

A 6-year-old Labrador retriever was presented with subcutaneous mass increasing in size in the past 15-20 days. On scrotal palpation, the dog was bilaterally cryptorchid and ultrasonography (Mylab Delta, Esaote pvt ltd., India) revealed an enlarged testicle as large mixed echogenic and cavitated within subcutaneous tissue, while the other could not be visualized. (Fig. 1). The decision was taken to remove the enlarged testicle surgically.

TREATMENT AND DISCUSSION

The dog was fasted overnight and pre-medicated with Atropine sulphate @ 0.03 mg/kg b. wt. and Xylazine @ 1.0 mg/kg b. wt. intramuscularly. Following aseptic precautions and under intravenous anaesthesia (Ketamine @ 5mg/kg b. wt. and Midazolam @ 0.2 mg/kg b. wt.), the enlarged testicular mass was removed following standard surgical procedure (Kudnig and Seguin, 2012). A 4-4.5 cm incision was made over the mass at the right ventral abdominal region. The narrow end of the mass was directed towards the incision to facilitate dislodgement (Fig. 2). Blood vessels in thickened spermatic cord were ligated at the base of testicular mass using Vicryl 2-0 and the mass was resected and checked for any bleeding. The absorbable gelatin sponge was placed to minimize the existing dead space. Incision closure involved simple continuous suture for subcutaneous tissue using Vicryl 2-0, followed by simple interrupted sutures of skin using Ethilon 2-0. Ceftriaxone @ 25 mg/kg b. wt. for 5 days and Meloxicam @0.3 mg/kg b. wt. for 3 days were given. The recovery was uneventful and sutures were removed after 15 days.

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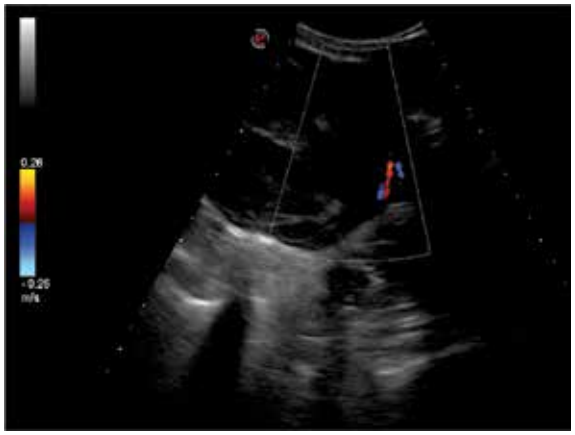

Fig. 1: Large mixed echogenic and cavitated testicle

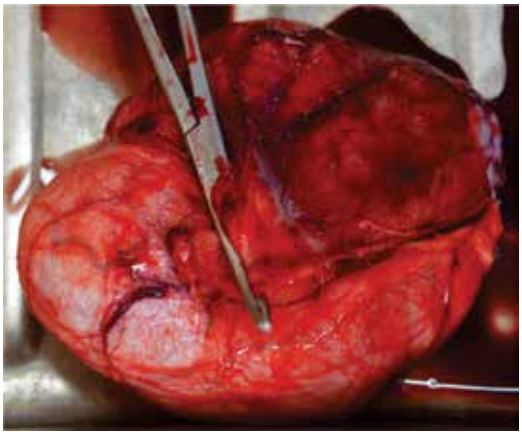

Fig. 2: Surgically removed intra-abdominal testicular mass

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CERVICOTOMY FOR THE MANAGEMENT OF DYSTOCIA DUE TO PREPARTUM VAGINO-CERVICAL PROLAPSE IN COW

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ABSTRACT

A case of dystocia due to peripartum vagino-cervical prolapse and imperfect cervical dilatation in a HF crossbred cow and it's per vaginal delivery by cervicotomy is reported.

Key words: Cow, Cervicotomy, Cervico-vaginal prolapse, Dystocia

INTRODUCTION

Ischemia of cervical region due to prepartum prolapse in a cow may be responsible for imperfect cervical dilatation, thus leading to difficulty in normal expulsion of fetus even with normal presentation, position and posture of fetus. In such cases, cervicotomy is the simple procedure for alleviating the condition compared to cesarean section (Noakes *et al.,* 2009). The present report describes a case of dystocia due to vagino-cervical prolapse coupled with imperfect cervical dilatation and its successful management by cervicotomy in a HF crossbred cow.

CASE HISTORY AND OBSERVATIONS

A HF crossbred cow (age, 4.6 yr; b. wt., 300 kg) was presented with the history of straining and was unable to deliver the full-term calf for past 3 h. The animal was in lateral recumbency and clinical examination of the cow revealed edematous vulva with congested vaginal mucus membrane. A torn snare tied to fetal extremities present in vaginal passage revealed an earlier handling of the case. Further vaginal examination explored a dry vaginal passage with stenosed and partially dilated (3 fingers) cervical rim. The fetal limbs were in external os of cervix and remaining fetal parts were not palpable.

TREATMENT AND DISCUSSION

Treatment was initiated with intravenous administration of 3.5L 5% dextrose normal saline. Carboxymethly cellulose gel was infused through available space in cervix along with fanning of cervical os. After 40 min of fanning, there was no progress in cervical relaxation. The cow was subjected to low caudal epidural anaesthesia (1ml 2% lignocaine HCl/ 50kg b. wt.). Thereafter, a snare was applied to fetal extremities followed by mild traction, but this lead to prolapse of entire cervix. Hence, it was decided to relieve the fetus by cervicotomy.

For cervicotomy, mild traction force was applied to place the cervix in a position so that the incision can be made at 10'O clock position involving only the circular muscles. Consequently, the cervical dilatation was sufficient enough to deliver the live male calf by slight traction. The cervical incision was sutured by continuous interlock pattern with absorbable catgut (size - 2) and was replaced in position (Fig. 1). Thereafter, the cervix was lavaged with warm saline to remove the debris and cetrimide cream was applied over cervix and vaginal region. The prolapsed mass was reduced and simple vulval tape retention suture was applied as preventive measure for recurrence. The dam was treated with routine parenteral antibiotic plus supportive therapy for the next five days. The

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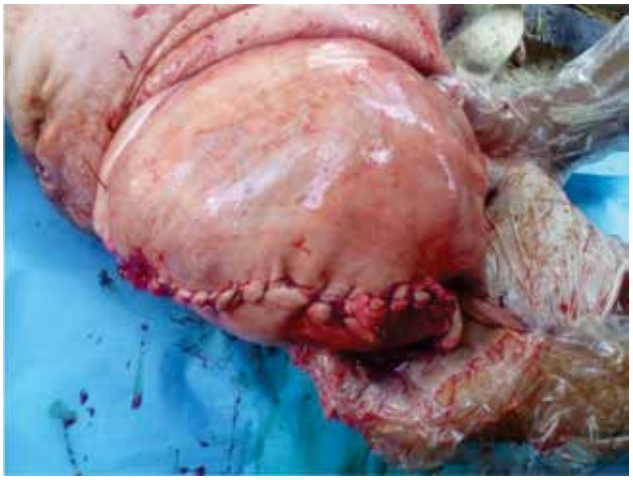

Fig. 1: Cervicotomy followed by cervical suture in a cow

examination of animal after a week revealed an uneventful recovery.

In cows, vaginal prolapse is usually a chronic, hereditary, recurrent, pre or peripartum condition and most commonly occurs in heavy dairy cattle before calving, usually in third trimester of pregnancy (Noakes *et al.,* 2009). Cervicotomy as a management technique in a fresh case of imperfect cervical dilatation was reported earlier in cow (Sathiamoorthy *et al.,* 2011). In brief, cervicotomy is a simple and effective method for approaching a case of imperfect cervical dilatation in cow.

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MEDICAL MANAGEMENT OF A CLOSED TYPE PYOMETRA USING MISOPROSTOL AND CLOPROSTENOL COMBINATION IN A PUG

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ABSTRACT

A female pug had the history of dullness, depression, inappetance for one week and was crossed 20 day earlier in her first heat. The clinical examination revealed mild congested and dry vaginal mucus membrane without any vaginal discharge. Ultrasound examination revealed multiple anechoic sacculations. The case was diagnosed as closed cervix pyometra and was treated with Misoprostol and $PGF_{2\alpha}$ combination. The female pug showed an uneventful recovery subsequent to treatment.

Key words: Bitch, Closed cervix pyometra, Misoprostol, Pug, $PGF_{2\alpha}$

INTRODUCTION

Pyometra is a hormonally mediated reproductive disorder that affects sexually intact mature bitches before they reach the age of 10 year (Baithalu *et al*., 2010). This disease generally occurs during or immediately following a period of progesterone dominance (Blendinger *et al*.,1997). The elevated progesterone in diestrus period leads to suppression of uterine contractions, an increase in endometrial gland secretions, closure of cervix, and suppression of immune response, thus creating a favourable environment for bacterial growth. It can be acute or chronic systemic disorder with presence (open cervix pyometra) or absence (closed cervix pyometra) of vaginal discharge; of which, the later being a medical emergency requiring rapid intervention to prevent subsequent sepsis and potential patient death (Baithalu *et al*., 2010).

CASE HISTORY AND CLINICAL OBSERVATIONS

A 11-month-old female pug had the history of dullness, depression, inappetance for one week and was crossed 20 days back in her first heat. The clinical examination revealed body temperature (101.6°F) and pulse rate (110/min) to be within a normal range. The vaginal mucus membrane was mildly congested and dry without any vaginal discharge. On ultrasound examination, multiple anechoic sacculations were noticed and blood picture revealed leucocytosis (neutrophilia). On the basis of history, clinical examination and haematological report, the case was diagnosed as closed cervix pyometra.

TREATMENT AND DISCUSSION

Ovariohysteroctomy is the choice of treatment for pyometra in older and non-breeding bitches. If the condition is not life threatening and the animal is young, then valuable restoration of fertility may be done with medical management using prostaglandin. However, the use of prostaglandins in closed pyometra is contraindicated. In the present case, as the dog was young and the owner was interested in future breeding, medical management with Misoprostol (prostaglandin E_2) @ 400µg total dose pervaginum was initiated in an attempt to relax the cervix prior to $PGF_{2\alpha}$ administration and the treatment was repeated 48h later. A foul smelling purulent vaginal discharge was reported 48h later and thereafter, the dog was treated with Inj. $PGF_{2\alpha}$ (Cloprostenol) @ 2.5µg/kg b.

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wt., S/C, once daily for 7 days. In order to minimize the side effects of $PGF_{2\alpha}$, atropine sulphate (0.02 mg/kg b. wt.) was administered S/C prior to $PGF_{2\alpha}$ inj. In addition, Tab. Amoxicillin and Clavulanic acid @ 20 mg/kg b. wt. PO was administered twice daily for 14 days. Ultrasound examination on day 7 revealed mild anechoic sacculations but the dog was active and feeding habits had improved. The review on day 14 post treatment revealed no uterine involvement suggesting complete response to $PGF_{2\alpha}$ administration.

In bitches, the incidence of pyometra was reported as 9.0-15.2% (Pretzer, 2008) with higher incidence noticed in smaller breeds (Shiju Simon *et al*., 2011). The exogenous or endogenous concentration of circulating steroid hormone particularly estrogen and progesterone influence the distribution of steroid receptors within the uterus. The regulation of estrogen and progesterone receptors in endometrial glands plays an important role in pathogenesis of pyometra complex in bitch (Baithalu *et al*., 2010). The administration of prostaglandin E_2 pervaginally leads to remodelling of cervical extracellular matrix (Ledger *et al*., 1983) and relaxation of cervix while prostaglandin F2 increases myometrial contractility, regression of corpus luteum and, thereby causing expulsion of uterine contents.

In conclusion, the combination of Misoprostol and $PGF_{2\alpha}$ can be used to manage closed pyometra in young and clinically active bitches in order to restore future fertility of bitches.

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SUCCESSFUL MANAGEMENT OF A VIOLENT ASIAN ELEPHANT IN MUSTH UNDER XYLAZINE ANAESTHESIA

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ABSTRACT

Successful management of a violent male Asian elephant (*Elephas maximus*) in musth was carried out using 700 mg Xylazine HCl to obtain desired standing anaesthesia. The legs of elephant were tied with chains and tethered by using special jute rope to prevent his movement until the musth period was over. In brief, better awareness of people on physiological behavoiur of elephants for detection of musth as well as to adopt appropriate management procedures is referred.

Keywords: Anaesthesia, Elephant, Management, Musth, Xylazine

INTRODUCTION

Musth is similar to rutting behaviour in ungulates and is associated with periodic increases of testosterone secretion. Most matings occur in this period (Poole, 1989). During musth, the animal becomes more restless, energetic, aggressive and generally irritable and oversensitive to sound and movements. Musth is generally a normal phenomenon but it becomes dangerous in case of man-elephant interactions (Rajaraman, 2006). During musth, the elephants are uncontrollable and become a risk to human life and property. Therefore, this condition needs immediate veterinary interventions for its better management.

CASE HISTORY AND OBSERVATIONS

A male Asian elephant of (Age, 40 yr; b. wt. 4000 kg) was reported as violent and destructive in Tekang village, East Siang district of Arunachal Pradesh. The owner reported that elephant injured his *mahout* and was on the run of 21 days destroying and damaging houses in villages. Also, the elephant charged to everyone as soon as he got the smell of any nearby human. The owner approached the local forest department for veterinary interventions. The elephant was detected to be in musth on the basis of clinical signs. It was finally decided to chemically restrain the elephant and to tie his legs with chains to restrict the movements until the musth period is over.

A total volume of 7 ml or 700 mg Xylazine HCl was loaded in a metal dart and projected through Dist-inject N 60 rifle to the rump region. As the elephant had the tendency to charge everyone, we had to fire a gun shot in air simultaneously with firing the dart so as to safeguard ourselves and to deter the attention of elephant from charging. After the dart injection, the visual contact was lost as the site was in deep forest. The elephant was found with the signs of anaesthesia after 15 minutes of dart injection about 200 meter away from the site of darting. The legs of elephant were tied with metal chains and tethered him in a nearer tree to restrict his movement until the musth period is over. The elephant was in constant watch till recovered from the standing anaesthesia.

TREATMENT AND DISCUSSION

Xylazine HCl is extensively used in elephants as a popular sedative because of its high therapeutic index, smooth induction and recoveries (Sarma and Pathak, 2001).The use of Xylazine @ 0.1-0.2 mg/kg b.

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wt. is recommended for sedation in Asian elephants. However, in extreme aggressiveness, musth, painful conditions and ambient disturbances may necessitate higher doses (Cheeran *et al*., 2002). After 15 minutes of the dart injection, the elephant manifested with signs of standing anaesthesia. After 20 minutes, complete standing anaesthesia was achieved and the signs of anaesthesia were recorded as diminished trunk, tail, restricted ear movement and relaxation of penis with dribbling of urine and loud breathing sound.

Once the musth elephant was completely sedated, all the legs were tied with chains and tethered with special jute rope to a nearer big tree for restricting his movements as reported earlier (Nigam *et al*., 2006). The anaesthesia lasted up to 3.5 h which was similar with the findings of others (Ahmed *et al*., 2017). The *mahout* was advised to keep constant watch on the elephant and not to remove the chains until the musth period is over. He was also instructed to provide reduced feed, sufficient clean water and to observe for any behavioral change. After 12 days, the elephant became uneventfully normal and with veterinarian instruction the chains were removed.

Musth appears to be similar to rutting behaviour which is associated with periodic increase secretion of testosterone. As it a normal physiological phenomenon among physically healthy bulls, it does not need any treatment. Musth is an emergency condition and might be the important cause for man-elephant conflicts as reported in the present case. Therefore, it needs special management which included chaining the animal away from contact with people and other elephants and withdrawal of feed to reduce the musth period by deteriorating physical health.

In the present case, chemical restraining and securing of musth elephant is adopted as the elephant became a threat to human life. This paper elaborates a successful chemical restraining of a violent elephant with Xylazine HCl anaesthesia in musth. We refer better awareness of people on physiological behavior of elephants for detection of musth as well as to adopt appropriate management procedures.

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MANAGEMENT OF DYSTOCIA DUE TO MACERATED FETUS IN A MARE

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ABSTRACT

The present case report puts on record successful management of a mare suffering from dystocia due to initial stages of maceration of fetus. A full-term pregnant seven-year-old mare was presented with the history of vaginal discharges followed by mild straining without any progress. The vaginal examination revealed a fully dilated cervix with the presence of a fetus with carpal flexion. The flexion was corrected and fetus was retracted successfully. The mare was administered fluid, analgesic and antibiotic parentally apart from intra-uterine drug treatment and the mare has resumed normal health.

Keywords: Carpal flexion, Dystocia, Fetus, Maceration, Mare

INTRODUCTION

Any fetal disposition other than anterior presentation, dorsal position and normal posture is likely to result in dystocia (Sane *et al*., 1994). The gestation period in the mare is 340 days, but foals with delayed development may be born normally up to 385 days and some mares may foal prior to 315 days (Davis Morel *et al*., 2002). Foaling in the mare is a violent and short process with the birth of foal completing within 30-70 minutes following the rupture of chorioallantoic membrane. Occurrence of dystocia in mares is comparatively less common but due foals having long neck and legs may assume many postures, which could cause problem during foaling (Narale *et al*., 2007).

CASE HISTORY AND OBSERVATIONS

A seven-year-old Marwari mare was presented with prior history of vaginal discharges 7 days prior to presentation at the clinic and general treatment given by local veterinarian. The mare was at 335 days of gestation and was intermittently straining, showing standing and sitting postures. There was discharge of blood tinged fluid from the vagina. The rectal temperature was 101.3°F, pulse rate 42 beats/min and respiration rate was 32 breaths/min. Mucous membrane was pink in color and hydration status was normal. On rectal examination, fetus was in anterior position and forelimbs were flexed from carpal joint and the neck was deviated laterally. Fetal membranes were putrefied and maceration of fetus had initiated.

TREATMENT AND DISCUSSION

The mare was placed in a dust free quiet area. Animal was kept in standing position with epidural anesthesia (8 ml 2% lidocaine) and general anaesthesia (10 ml Xylazine IV). The perineal area was washed with non-irritant antiseptic solution and 250 ml of liquid paraffin was pumped into the uterus with a sterile flexible plastic tube. The carpal flexion of both limb was corrected by mutation and ropes were applied at fetlock joint. A repulsion was attempted at shoulder joint to facilitate an approach to neck, which was corrected manually. Again, the lubrication was done with liquid paraffin, thereafter fetus was removed just by traction applied through rope snares. Uterine lavage with look warm normal saline and 2% betadine was performed, and 25 IU oxytocin in normal saline was also administered. Antibiotic therapy, anti-inflammatory drugs and fluid therapy was appropriately administered for 5 days.

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Fig. 1: Macerated Foal

The malposture of long fetal extremities is major cause of dystocia in mare although positional and presentational abnormalities occur to a lesser degree. In the present case, the cause of dystocia was fetal postural abnormality (flexion of carpal joint). Classically, the methods of dystocia correction were divided into assisted vaginal delivery, controlled vaginal delivery, fetotomy and caesarean section (Embertson *et al.*, 1995). In assisted vaginal delivery, the mare is aware and assisted to a small or large degree for vaginal delivery of an intact foal within 10-15 minutes (Embertson *et al*., 1995), Controlled vaginal delivery employs general anesthesia, if the foal cannot be delivered within 15 minutes a fetotomy (if the foal is dead) or caesarean section (if foal is live) was performed. In the present case, the forelimbs of fetus were flexed from carpal joint and neck was deviated laterally, therefore, normal and assisted delivery was not possible. Due to severe straining shown by mare during handling, the controlled vaginal delivery under general anesthesia with xylazine was performed.

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Renowned Veterinarian Dr. R.D. Sharma Passes Away

Dr. R.D. Sharma, Former Additional Director of Research (Veterinary and Animal Sciences) and Professor-cum-Head, Department of Veterinary Gynaecology and Obstetrics, Punjab Agricultural University born on 28 August, 1933 in a small town, Bhawanigarh, Punjab, left for his heavenly abode on 6 December, 2017. Dr. Sharma graduated (Bachelor of Veterinary Science & Animal Husbandry) from Punjab University in 1956, did his Masters from OHIO State University, Columbus, Ohio, USA in 1968 and Ph.D. (Veterinary Gynaecology and Obstetrics) from Punjab Agricultural University, Ludhiana in 1976. He initially worked as Veterinary Assistant Surgeon, Technical Assistant at Govt. Progeny Testing Farm, Hisar and later as Research Assistant (A.I.) at Department of Animal Husbandry, PAU, Hisar. He joined as Assistant Professor of Veterinary Gynaecology and Obstetrics at College of Veterinary Science, PAU, Ludhiana in 1969. Dr. Sharma remained Professor-cum-Head of the Department of Veterinary Gynaecology and Obstetrics for 14 years before taking over as Additional Director Research in 1991 till his superannuation in 1993. He also worked as Professor Emeritus for five years thereafter in the Department. He guided 12 M.V.Sc. and 09 PhD students as Major advisor and had more than 150 publications to his credit. Many laboratories for Obstetrics, Gynaecology, Andrology, Radioimmunoassay, Postgraduate Research and Cold Room for preserving fetuses for obstetrical training were established under his guidance because of which the department, later on, was recognized as Centre of Advance Faculty Training.

Dr. Sharma was a dedicated teacher and a clinician par excellence who had great love for animals. Owing to his selfless service to the animals and the farmers, he earned a lot of respect from the community. He was famous for his softspoken nature, ever helping attitude and dedication towards profession. Dr. Sharma had a passion for Obstetrics and could not bear the suffering of the animals. He therefore, established a 24 h clinical service in the Veterinary Hospital where the cases were attended even at odd hours. Keeping in view the contributions of Dr. R.D. Sharma towards society and science, Indian Societies for Study of Animal Reproduction started a prestigious award in his name for best scientific presentation in Obstetrics during the National Conventions. Many of his students are presently working on many eminent positions in India and Abroad. Sudden demise of Dr. R.D. Sharma has left a void which will not be fulfilled so easily.

REPORT OF INDIAN SOCIETY FOR STUDY OF ANIMAL REPRODUCTION (ISSAR) 2017

Indian Society for Study of Animal Reproduction (ISSAR) has organized 33rd Annual Convention of ISSAR and National Symposium on "Use of reproductive technologies and production improvement in livestock species aiming to socioeconomic development of rural mass" during February 9-11, 2018 at City of Joy, Kolkata. The symposium began with colourful inaugural session on February 9, 2018 which was inaugurated by Hon'ble Purnendu Basu, Minister of Technical Education, Training and Skill Development, Govt. of West Bengal in the presence of Hon'ble Vice–Chancellor of West Bengal University of Animal and Fishery Sciences, Prof. Purnendu Biswas, Director, Animal Husbandry and Veterinary Sciences, Govt. of West Bengal, Dr. (Capt.) A.G. Bandopadhyay, Chairman, West Bengal cooperative milk producer federation Ltd., Sri Paras Dutta and other dignitaries. A total of 409 delegates from different states of country participated. The entire symposium was spread over 8 technical sessions. A total of 12 invited lead speakers gave exhaustive presentations on various aspects of reproductive technologies covering most important livestock species. This year eight young scientists were selected for young scientist award on the basis of their performance during research paper presentations. Total 415 abstracts were included in this symposium. There were two special sessions; Dr. S.N. Luktuke Extempore presentation for young scientist (≤35 yr) and another for field veterinarian award i.e. clinical case presentation.

Glimpses of ISSAR 2017 Inaugural Session

Inaugurated by Hon'ble Purnendu Basu, Minister of Technical Education, Training and Skill Development, Govt. of West Bengal

Dr. Madhusudan Sarkar, Dr. J.L. Chakraborty with CEC, ISSAR members

Address by Hon'ble Purnendu Basu

Participation of ISSAR convention by delegates from different parts of the country

Release of Souvenir of 33rd ISSAR convention during inaugural session

ISSAR activity report by general Secretary, ISSAR Dr. Shiv Prasad

Address by Hon'ble president, ISSAR Dr. V. Chadrashermurty

C.R. Sane oration delivered by Dr. D. Kathiresan

Different scientific sessions held during convention

Poster session presentation by scientist during convention

ISSAR AWARDS 2017 - AWARDED DURING 33RD ANNUAL CONVENTION AT KOLKATA

Life Time Achievement Award: Dr. D. Kathiresan, Ex-Dean, College of Veterinary and Animal Sciences, Aizawl, Mizoram was honoured with Life Time Achievement Award-2017 of the society for his contribution to the field of Animal Reproduction for more than 3 decades

ISSAR Fellow - 2017: Dr. J.B. Phogat, Dean, Post graduate Studies, Lal Lajpat Rai University of Veterinary and Animal Sciences, Hissar, Haryana was awarded with Fellow, Indian Society for Study of Animal Reproduction

ISSAR Fellow - 2017: Dr. S.P.S. Ghuman, Professor &Head, TVCC, GADVASU, Ludhiana, Punjab was awarded with Fellow, Indian Society for Study of Animal Reproduction

Nil's Lagerlof Memorial Award; This award is given to young scientist who has published best paper in the field of Animal Reproduction in preceding year. This year it goes to Dr. M.K. Patel and co-authors Drs. R.S. Cheema, A.K. Bansal and V.K. Gandotra

Dr. G.B. Singh Memorial Award: This award is given to the young scientist who has published best paper in the field of Animal Reproduction in preceding year. This year it goes to Dr. N.K.J. Pandey and co-authors Drs. H.P. Gupta, Shiv Prasad and S.K. Sheetal.

Dr. N.C. Sharma award: This award is given to the scientist who has published best research paper in Indian Journal of Animal Reproduction in preceding year. This year it goes to Dr. Bilawal Singh and co-authors Drs. S.P.S. Ghuman, R.S. Cheema and A.K. Bansal

Dr. R.D. Sharma Award: This award is given to the scientist who has published best research paper in the field of Veterinary Obstetrics. This year it goes to Dr. N. Singh and co-authors were Dr. V.K. Gandotra, S.P.S. Ghuman, S.S. Dhindsa and P.S. Brar

S.N. Luktuke Extempore Award: This award is based on extempore presentation by young scientist below the age of 35 years on a specified topic during convention. This year it was won by Dr. Shilpa V.S. from Tamil Nadu.

INTAS quiz competition won by Dr. Meenakshi Rawat, C.V.A.Sc., GBPUA&T., Pantnagar, Uttarakhand.

Best Field Veterinarian Award: This award is given to the best field veterinarian on the basis of his presentation on clinical cases handled under field conditions. This year it goes to a very senior veterinarian Dr. K. Ganesan.

Young scientist winner of different scientific sessions during convention

PROCEEDINGS OF 33RD ANNUAL CONVENTION OF ISSAR HELD AT KOLKATA, FEBRUARY 9-11, 2018

General body meeting of ISSAR was held on February 10, 2018 in the evening and the following decisions were taken.

1. It was approved by GBM that Rs 10,000 (Rs. Ten thousand) per issue as demanded by www.indianjournal.com for uploading of our journal (IJAR) is viable solution as it will be free of cost for all the members for downloading of their papers or other materials.
2. GBM approved a cash award of Rs. 2,100 per winner scientist (8) instead of travel grant.
3. Financial status and expenditure presented by treasurer, ISSAR was approved by GBM.
4. CEC of ISSAR has expressed great concern about poor reporting of the state chapters about their financial status, executive body elections and technical activities conducted in the state and any significant achievement of the members at national or international level. State secretaries were impressed upon to collect, compile the information and report to General Secretary, ISSAR for wider dissemination.
5. The dates to apply for various awards of ISSAR remain unchanged i.e. 31st March of every year.
6. It was decided in GBM that mid convention meeting of CEC is compulsory for shaping the activities and making it more meaningful. CEC should meet preferably at the venue of next proposed convention.
7. From the next year R.D. Sharma Award shall be known as R.D.Sharma Memorial Award.
8. The next convention of ISSAR which was decided in GBM for Rewa (M.P.) has been changed to College of Veterinary and Animal Sciences, Anand (Gujarat) by CEC after consultation looking into various aspects.
9. For reviewing of ISSAR by-laws, a committee under the chairmanship of Dr. K. Kulasheker was constituted with Dr. Shiv Prasad, Dr. M. Selvaraju, Dr. F.A. Ahmad, Dr. M.K. Tandley and Dr. D.K. Nandi. They will complete the work within 3 months.

Scientific recommendations of 32nd Annual Convention of ISSAR and National Symposium on "Use of reproductive technologies and production improvement in livestock species aiming to socioeconomic development of rural mass" held at West Bengal University of Veterinary and Fishery Sciences, Kolkata w.e.f. 9-11 February, 2018.

1. Use of ultrasonography should be encouraged under field conditions for diagnosis of various reproductive ailments and also for pregnancy diagnosis.
2. Development of diagnostic tools for various reproductive disorders also needs fresh attention.
3. Molecular markers for selection of breeding bulls at early stage should be established for indigenous cattle and buffaloes.
4. Infrastructural boosting is required to reach up to end users. Elite bull semen should only be allowed to be used for breeding.
5. Frozen semen from elite indigenous breeds should also be given good attention.
6. Regular training on obstetrical techniques should be conducted to acquire skill in the field of Obstetrics by using various models.
7. Research on sexing of spermatozoa for skewing of sex ratio needs to be given priority.
8. Research work on embryo biotechnology and stem cell technology should be enhanced and it should continue.
9. Research work on equine reproduction should also find place in veterinary colleges of the country as in domestic animals.

APPLICATION FOR LIFE MEMBERSHIP IN THE INDIAN SOCIETY FOR STUDY OF ANIMAL REPRODUCTION (REG. NO. F 5156/MUMBAI)

Name	:
Address	:
Professional qualification	:
Field of specialization	:
Address to which all correspondence should be made (with Pin Code)	:
Telephone No. Home Office	: :
Mobile No.	:
e-mail address	:
Whether Membership is direct or through State Chapter	:
Details of remittance for life membership fee (Cash / DD / NEFT / RTGS)	:
Place	:
Date	:

Signature of the Applicant

Note:

- Life Membership fee - Direct application Rs.1510 (Rs One thousand five hundred and ten only)
- Life Membership fee - Through state chapter Rs. 1135 (Rs One thousand one hundred and thirty five only)
- The fees may be drawn as the cheque / demand draft in favour of The Treasurer, ISSAR, payable at Namakkal or fees may be sent through NEFT / RTGS for the following account. Name: Treasurer, ISSAR, Name of the Bank: Union Bank of India, Branch Name: Namakkal, Account number: 548802010014809, IFSC code: UBIN0554880, MICR code: 637026002.

AUTHOR GUIDELINES

General

The Indian Journal of Animal Reproduction (IJAR) is the official organ of the Indian Society for the Study of Animal Reproduction (ISSAR) and publishes basic/applied research articles, short communication and case reports in the field of Veterinary Gynaecology, Obstetrics, Andrology, Semenology, Artificial Insemination, Embryo Transfer and other Assisted Reproductive Technologies. The journal is published biannually i.e. June and December. The manuscripts are subjected to a thorough peer-review by referees and journal publishes only well-written manuscripts contributing towards scientific and practical advancement in the field of reproduction. The publication language of journal is English. The manuscripts are accepted for publication with the understanding that they have not been published, submitted or accepted for publication elsewhere in any language. The manuscript will be accepted after editorial revision based on comments. The manuscript found unsuitable for publication in the IJAR will be communicated to the author and no correspondence will be entertained in this regard. The decision of the editorial board shall be final. The responsibility with regard to the data and technical details of the article would be with the authors. The copyright of manuscripts, accepted for publication, rests with ISSAR.

Manuscript submission

The manuscript submission and review process is handled online. To submit an article to The Indian Journal of Animal Reproduction, please go to *http://www.indianjournals.com/ijor.aspx?target=ijor:IJAR3&type=home* and please ensure that the corresponding author's valid email is present in the manuscript. Authors submitting a manuscript need to upload online Author's Declaration Certificate along with the manuscript as a supplementary file. The research article, short communication or case report should be strictly formatted as mentioned below.

Review article: The length including figures, tables and references, should not exceed 4,000 words. The standard format of review article shall include abstract, keywords, review and references. Other guidelines as mentioned in original research article shall be followed. The main focus of review should be the research carried out by the authors in India.

Research article: The length including figures, tables and references, should not exceed 2,000 words. The standard format of original research article must be as per the example given in the box below. The manuscript must be computer typed (Arial, font 12) with single spacing using standard software (Microsoft Word). Margins should be 2.54 cm on Top and Bottom and 3.17 cm on Left and Right. Title should be concise, informative and containing keywords necessary to enable retrieval by search engines. Abstract should limit to maximum 150 words. The abstract should not just contain repeated/rewritten results. Efforts should be made to give principal significant findings only and concluding the findings keeping in view the importance of work done. The abstract should be self-explanatory to generate interest in the reader for reading/citing the article. Keywords should be five and alphabetically arranged. Introduction should be related to parameters done in the study and justifying the importance of work carried out. Materials and Methods should be described in sufficient detail (animal management, methods, statistics etc.) to allow for repetition of the experiments. Results and Discussion should focus on the interpretation of findings and repetition of data in text, already presented in table should be avoided. Figure and Table number should be kept to a minimum. Tables should be elegantly made, in a vertical format and within the page margins. Photographs or drawings must be sharp, of high contrast and to be submitted in JPEG format. All the figures and tables should be referred in the text.

EARLY LUTEAL PHASE HORMONAL TREATMENT FOR INCREASING CONCEPTION RATE DURING SUMMER SEASON IN BUFFALO

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ABSTRACT

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INTRODUCTION

MATERIALS AND METHODS

RESULTS AND DISCUSSION

ACKNOWLEDGEMENTS (if any)

REFERENCES

Table: (if any)

Figure: (if any)

References: Citations in the text should be given by placing in parenthesis the name(s) of author(s) and the year of publication, e.g. (Rao, 1996), (Mann and Lamming, 2001), (Binelli *et al*., 2001), (Rao, 1996; Binelli *et al*., 2001 and Mann and Lamming, 2001). Avoid writing citations in text as Rao (1996) reported……, Binelli *et al*. (2001) found……. All publications cited in the text should be presented in the form of list arranged alphabetically according to author's surnames. The number of references should be restricted to maximum 15. The examples are given for citing a journal article and a book (chapter). For conference proceedings, include the name(s) of the editor(s) of the proceedings, the publisher and the place of publication. References of thesis and dissertations more than three year old should be avoided.

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