LAPAROSCOPIC CHROMOPERTUBATION FOR THE EVALUATION OF TUBAL PATENCY IN DAIRY CATTLE

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ABSTRACT

The present study was conducted to evaluate feasibility of laparoscopic chromopertubation using methylene blue dye as a tubal patency test in bovines. This test was done during routine laparoscopic examination of dairy cattle (n=5). Laparoscopy was done through left flank and blood / serum samples were collected for hematology, biochemical and hormone analysis at pre- (1 h) and post- (1 and 24 h) laparoscopic procedure. For evaluating tubal patency, Foley's catheter was inserted into the right uterine horn, fixed and about 30-40 ml autoclaved dilute methylene blue dye (2.5%) was then administered into the horn. The passage of dye through the fallopian tube was observed and appearance of blue color in the oviduct and spillage of dye through the fimbrial end was indicative of patency. The same procedure was repeated on left uterine horn to determine patency. The procedure was successfully done in four cattle and out of these, the left oviduct was blocked at utero-tubal junction only in one cattle. Pre- and post-laparoscopy, the hematological and serum enzyme parameters remained similar ($p>0.05$), however, serum cortisol was elevated at 1 h post-laparoscopy ($p<0.05$), which reached pre-laparoscopy concentrations by 24 h post-laparoscopy. Per-rectal examination about two months after procedure revealed uneventful recovery with no adverse impact in genital tract. To our knowledge, it is the first report on laparoscopic chromopertubation for tubal patency evaluation in bovines. In conclusion, laparoscopic chromopertubation using methylene blue dye is safe and innocuous procedure and can be used for evaluation of tubal patency in bovines in the future, but it needs further study and validation especially for impact on fertility.

Keywords: Bovines, Cattle, Chromopertubation, Laparoscopy, Tubal patency

INTRODUCTION

The oviduct is an essential component of normal reproduction, providing a path for the oocyte and sperm to reach the site of fertilization and subsequently for the embryo to reach the uterus. The abnormalities of fallopian tubes are one of the most important causes of female infertility in all species. The clinical diagnosis of uterine tube abnormalities by rectal palpation is only possible if there is gross enlargement and thickening of the tube, but less severe abnormalities and their patency are not detectable by rectal palpation and can only be identified by special techniques. Such lesions of fallopian tubes generally result in tubal occlusion and the tubes lose patency either unilaterally or bilaterally (Johari and Sharma, 1964). An early diagnosis of this condition is essential for suitable course of action for the economic reason.

Laparoscopy with chromopertubation is widely accepted as the '*gold standard*' method for evaluating the tubal patency and is presently considered the most accurate diagnostic test available for evaluating tubal-related sub-fertility in humans (Dwivedi *et al.,* 2012; Shetty *et al*., 2013). The procedure is usually done during a laparoscopy for infertility work-up, where a colored dye is passed through the fallopian tubes to confirm their patency. Currently, the tubal patency tests used in bovines are not accurate, especially for the evaluation of individual patency. Further, oviductal blockage was mentioned mostly from abattoir studies and the diagnosis in live animals continues to be difficult and less reliable. Therefore, the present study was conducted to evaluate the feasibility of

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laparoscopic chromopertubation using methylene blue dye as a tubal patency test in dairy cattle.

MATERIALS AND METHODS

Laparoscopic chromopertubation was done in five dairy cattle to assess the patency of fallopian tube. To reduce ruminal contents and to facilitate examination of genital tract during laparoscopy, the feed was withheld, but not water, for a minimum period of 24 h. Laparoscopic instruments (KARL STORZ Gmbh and Co. KG, Tuttlingen Germany) were sterilized by chemical method using 2% glutaraldihyde solution w/v.

Before laparoscopic procedure, each cattle was injected anti-inflammatory drug (Meloxicam 0.3 mg/kg BW) and antibiotic (Streptopenicillin 2.5g im) as a pre-operative treatment. The animal was restrained in standing crush designed for laparoscopy and was sedated with xylazine (0.05 mg/kg BW, im). The left flank was prepared aseptically, draped and local infiltration was done with lignocaine (2%) at laparoscopic and instrument ports. A sharp trocar along with cannula (6 mm) was inserted through a small skin incision given with BP blade (No. 10) into the peritoneal cavity at 8-10 cm cranial to the tip of tuber coxae and 6-8 cm ventral to the transverse processes of lumbar vertebrae at the junction of middle and caudal third flank. Once the trocar entered the peritoneal cavity, it was removed and cannula was pushed deep into abdominal cavity for CO_2 insufflation up to 5 mm Hg. The 6 mm cannula was replaced by 11 mm cannula for insertion of telescope into the peritoneal cavity. After optimum pneumoperitoneum was achieved (8 mm Hg), a 0° viewing angle laparoscope (10 mm, 57 cm) was inserted through the cannula and the tract was searched and visualized on the monitor. Similarly, instrument port was made ventral (18-20 cm) to the tuber coxae using 6 mm cannula followed by introduction of instrument through the cannula under endoscopic guidance. The palpation probe was used to manipulate the genital tract for complete examination of oviduct.

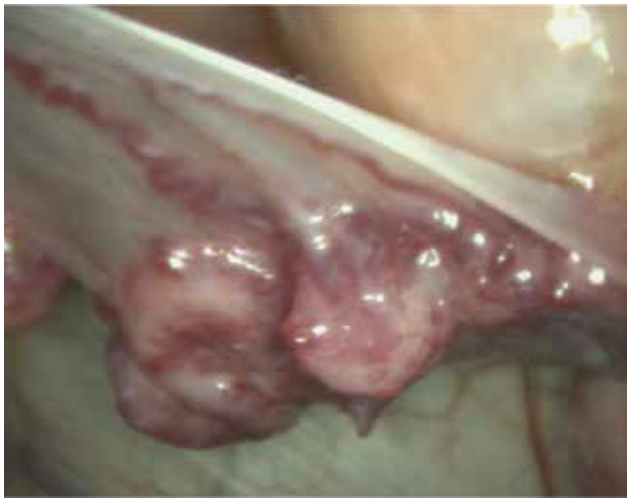
For evaluating tubal patency, 2.5% methylene blue trihydrate dye was prepared in distilled water and autoclaved. Animal was given caudal epidural anesthesia using 2% lignocaine and the perineum was washed. Cervix was grasped through rectum and was dilated with the help of cervical dilator to facilitate insertion of Foley's catheter (18 Fr, 30 ml) into the uterus. After dilation of cervix, Foley's catheter was inserted into the right uterine horn with the help of stilet and was fixed in the cranial part of horn by inflating the balloon with syringe. Methylene blue dye of about 30-40 ml was then administered into the horn through Foley's catheter and the flow of dye through the fallopian tube was observed with the help of laparoscope. The appearance of blue color in the oviduct and spillage of dye through the fimbrial end was indicative of patency. Same procedure was repeated on left uterine horn to determine patency of left oviduct. After the completion of dye test, Foley's catheter was then deflated and removed. After complete examination, the instruments and telescope was removed and CO_2 was allowed to escape through the portals. The incision sites were sutured with one or two interrupted sutures. The peritoneum and abdominal muscles were closed using catgut (2 No.) and the skin was closed with silk (2 No.). The wound was dressed with povidone-Iodine solution and lorexane cream was applied topically. The post-operative care included daily monitoring of animals regarding behavior, feed and water intake and use of anti-inflammatory drug and antibiotic for 5 days. The wound was dressed with povidone-Iodine solution and lorexane cream for 7-10 days. The sutures were removed after 10-12 days of laparoscopic procedure.

Blood samples (n=5) were collected in vials containing EDTA at pre- (1 h) and post- (1 and 24 h) laparoscopic procedure for hematological analysis. Complete blood count was done immediately in automatic hematology analyzer (Mindray; BC-2800Vet). At same time points, serum samples were collected and stored in deep freezer at -20°C. Commercially available ELISA kits were used to assay

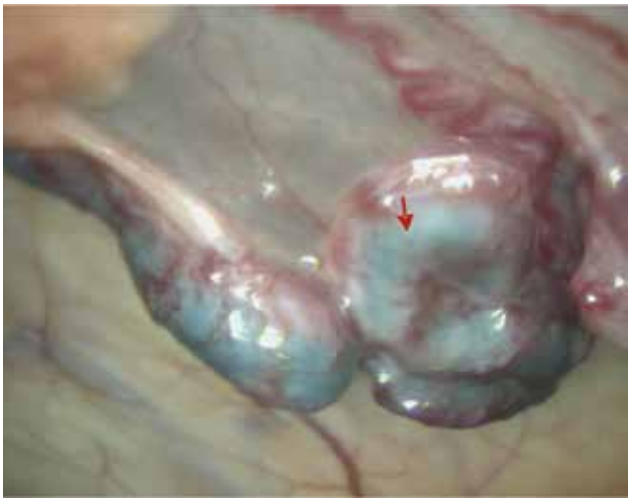
serum cortisol (DRG Instruments, GmbH, Germany). Serum enzymes (ALT, Alanine transaminase; AST, Aspartate transaminase; AP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase) and Total protein (TP) were measured by colorimetric method using semi-automatic biochemistry analyzer (Photometer 5010, ROBERT RIELE GmbH & Co, KG, Germany) with commercially available kits (AGGAPPE DIAGNOSTICS LTD, Kerala, India). The data was analyzed using statistical software SPSS version-16. The data with three variables was analyzed with ANOVA and the significance was determined by LSD and Duncan's test.

RESULTS AND DISCUSSION

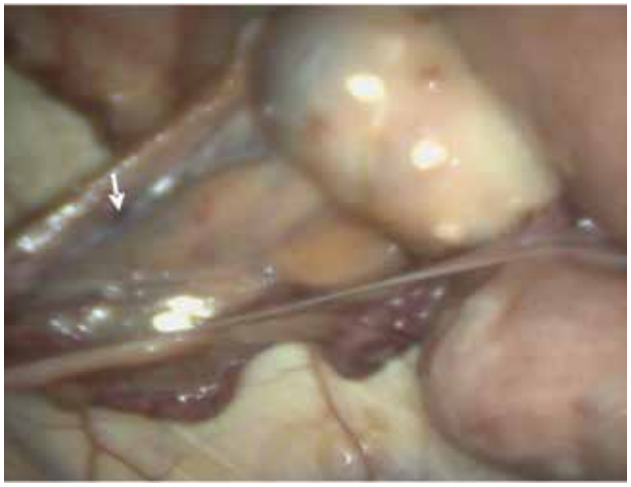
Laparoscopic chromopertubation using methylene blue dye (2.5%) for evaluation of tubal patency was successfully done in four out of five animals. In one animal, it became impossible to insert dye into the uterine horn due to fibroses of cervix. Nevertheless, out of four animals, left oviduct was blocked in one animal at utero-tubal junction as dye could not pass



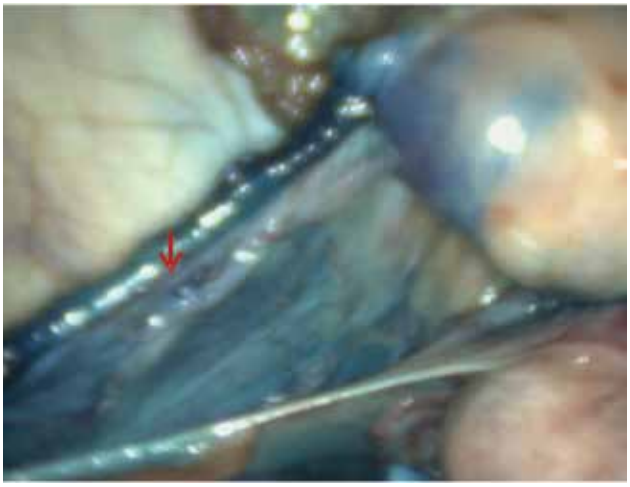
a) Fallopian tube before dye test



b) Fallopian tube after dye test (blue color)



c) Fimbrial end of oviduct before dye test



d) Fimbrial end of oviduct after dye test

Figure 1: Images showing passage of methylene blue dye during laparoscopic chromopertubation

beyond that point and there was enlargement of tubal part at the blockade point due to flow of dye from the uterus.

Laparoscopic chomopertubation for tubal patency evaluation has not been reported in animals. In human females, these procedures are routinely used for tubal infertility evaluation and recommended as first step in the investigation of infertile women with tubal factor (Shetty *et al.,* 2013; Tomar *et al*., 2014; Rezk and Shawkey 2015). Out of cases (n=100) of female infertility in which tubal patency was tested by injecting 0.5% methylene blue dye (10-15 ml) though the uterine cavity and observing its spillage from the fimbrial end of the fallopian tube, the most common pathology was observed as tubal blockage with (15%) or without adhesions (23%; Tomar *et al*., 2014). In a human female, central and peripheral cyanosis was observed after laparoscopic chromopertubation with 30 ml of 1% methylene blue (Dhanpal and Joseph, 2006). In the present study, no complication was observed in any cattle. Further, no acquired gross abnormality associated with dye test, like adhesions, was observed by manual examination after two months in the present study that was in contrast to earlier reports (Gul *et al.,* 2000).

The hematological parameters at pre- and post-laparoscopic examination were almost similar ($p>0.05$, Table 1). However, RBC's count, Hb, HCT and MCV increased consistently at post 1h and 24h compared to 1h before laparoscopy procedure, which may be due to compensatory physiological response to the CO_2 insufflation as it leads to temporary hypercarbia/ hypercapnia and metabolic acidosis. The results of hormone and enzyme analysis (Table 1) revealed an increase ($p<0.05$) in serum cortisol from 1h pre- to 1h post-laparoscopic examination, however, it decreased ($p>0.05$) at 24h post-laparoscopic examination. In accordance with the present findings, there was transient rise in plasma cortisol in ewes undergoing laparoscopic examination (Martin *et al*., 1981).

Table 1: Haematology, serum hormone and serum enzyme analysis of dairy cattle (n=5) at pre (1 h) and post (1 h, 24 h) laparoscopy

Parameter	Pre 1 h	Post 1 h	Post 24 h
WBC, x10^6/ml	12.24±2.06	14.18±3.50	9.73±0.62
Lymphocyte, x10^6/ml	5.25±1.16	7.45±2.67	6.20±1.71
Monocyte, x10^6/ml	1.04±0.18	1.23±0.21	0.89±0.17
Granulocyte, x10^6/ml	7.33±2.31	8.30±3.46	5.21±1.20
RBC, x10^6/ml	5.95±0.31	6.41±0.38	6.97±0.51
HGB, g/dl	9.49±0.65	10.22±0.82	10.91±0.9
MCV, fL	47.86±1.76	47.95±1.79	48.9±1.75
MCH, pg	16.0±0.98	16.1±1.0	15.7±0.9
Platelet, x10^6/ml	181.6±33.2	190.1±68.8	186.6±50.2
Cortisol, ng/ml	0.93±0.08^{ac}	1.21±0.07^{bc}	1.12±0.05^{cab}
ALT, U/L	30.9±3.9	32.6±4.2	39.7±4.7
AST, U/L	79.8±10.7^{ab}	88.4±9.6^{ba}	134.7±10.8^{c}
AP, U/L	70.0±8.4	71.1±11.1	79.5±4.8
GGT, U/L	10.8±1.0	11.6±1.2	11.7±1.4
Total Protein, g/dl	6.99±0.3	6.83±0.3	6.78±0.1

Means within a row with different superscripts differ significantly ($p<0.05$); ALT, Alanine transaminase; AST, Aspartate transaminase; AP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase; TP, Total protein

Similarly, serum AST was high ($p>0.05$) at 1h post- and further increased ($p<0.05$) at 24h post-laparoscopy compared to 1h pre-laparoscopic examination (Table 1). These findings are in agreement with earlier reports (Anderson *et al.,* 1993; Maiti *et al.,* 2013). All the animals recovered without any complication.

It was concluded that laparoscopic chomopertubation using methylene blue dye can be used for the evaluation of tubal patency in bovines. It is a safe and innocuous procedure but it needs further study and validation especially its impact on fertility.

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