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Therapeutic management of infectious repeat breeder buffaloes using herbal preparations

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Abstract
The study was conducted to determine the effect of herbal preparations as a therapy for repeat breeding syndrome in 24 buffaloes. White side test was carried out to check the possibility of sub-clinical genital infection in these animals. The repeat breeder buffaloes which were found to be positive for White side test were divided randomly into two equal groups (n=07) and control group (n=10). The infectious buffaloes Group I received Exapar-N® 100 ml, Janova® and Mintrus® and Group II Exapar-N® 50 ml, Janova® and Mintrus®. The Vaginal Electrical Resistance (VER) values of pregnant (242±6.27) and non-pregnant buffaloes (262.04±7.07) differ significantly (P<0.05). The color of estrual mucus in repeat breeder buffaloes was 23.27% transparent, 38.63% translucent and 34.09% whitish. 36.36%, 43.18% and 20.45% of repeat breeder buffaloes were having thick, viscous and thin consistency of estrual mucus. The mean pH value was 8.45±0.10 in infectious repeat breeder buffaloes. Cytology of uterine lavage revealed that, herbal uterine cleanser and restorative preparation (Exapar-N®) is efficacious in reducing the uterine infection. Out of 7 repeat breeder buffaloes 57.14% conceived with 2.43 services per conception in group I while, 14.28% conceived with 3 services per conception in group II.

Keywords: Repeat breeder buffaloes, PMNs, Vaginal Electrical Resistance (VER), estrual mucus

Introduction
Buffaloes are called as black gold of Indian farmers because of their adoptive nature in harsh climatic conditions, endowed tolerance to diseases and better survival under poor feeding and management practices. India has more than 105 millions of buffalo population that constitutes 57% of the total world buffalo population and India stands number one across the globe in milk production and of which more than 60% buffaloes alone (18th Livestock census, 2007) contribute milk. Currently, the incidence of infertility relatively increased with consequent reduction of productivity of farm animals. Ovarian inactivity, silent heat, endometritis and repeat breeding are the main reproductive disorders in buffaloes. Repeat breeding is a major problem adversely affecting the productive and reproductive performance in buffaloes. The repeat breeding syndrome is defined as a condition in which dairy animal have a regular estrus cycle and appear normal on superficial clinical examination but fail to become pregnant following three or more breeding (Bartlett *et al*.,1986) [1]. Repeat breeding results in delayed conception and longer inter-calving period and thus, reduces the economy of the dairy industry. The incidence of repeat breeding varies from 15-32% and seems to be lower in buffaloes kept individually on small-holdings than in large herds (Dhami *et al.*, 2009) [].2 Previous work has indicated that, delayed ovulation (Erb *et al.*, 1976) [3], inadequate luteal function (Kimura *et al.*, 1987) [4], embryonic death (Ayalon, 1984) [5], management errors (O' Farell *et al.*, 1983) [6] and non-specific uterine infection (Samad *et al.*, 1984) [7] are among some possible causes of repeat breeding. However, failure of fertilisation and early embryonic death are the two major groups responsible for repeat breeding (Tanabe and Casida, 1949) [8].

Materials and Methods
The study was carried out in 24 repeat breeder buffaloes belonging to members of Bidar-Gulbarga Milk Producers Union, buffaloes presented to VGO-OPD, Veterinary College, Bidar and APMC Veterinary Hospital Bidar. The buffaloes, which failed to conceive to 2 to 3 or more insemination using good quality frozen semen / served by healthy fertile buffalo bull at regular cycling estrus without any reproductive tract abnormalities were selected for the study.

These buffaloes were diagnosed for repeat breeding on the basis of history obtained by the animal owner (age, parity, number of AI / natural service done, history of dystocia / abortion / RFM, regularity of estrous cycle), per rectal examination, white side test and records.

Vaginal electrical resistance (VER)
VER was tested by estrus detector[2] on the day of estrus in all the repeat breeding buffaloes and values were recorded.

Physical characteristics of estrual mucus
Following confirmation of estrus, the cervico-vaginal mucus from buffaloes was collected aseptically in a sterile vial for studying various physical parameters / tests viz. color, consistency, pH, spinnbarkeit, arborization and white side test.

1. **Color:** Color of cervico-vaginal mucus was observed and graded as transparent, translucent and whitish.
2. **Consistency:** Consistency of cervico-vaginal mucus was observed and graded as viscous, thin and thick.
3. **pH:** The pH of cervico-vaginal mucus was recorded using universal indicator pH paper.
4. **Spinnbarkeit value:** A drop of cervico-vaginal mucus was spread on a clean glass slide and was lifted with the help of another glass slide along the side of a scale fixed vertically. The point of breaking up of mucus while lifting is the spinnbarkeit value recorded in centimetres.
5. **Arborization pattern:** A drop of cervico-vaginal mucus was spread on a clean glass slide and air dried then observed under low power objective under microscope for appearance and presentation of crystallization pattern. The type of arborization was classified as per Vadodaria and Prabhu, (1989) [9].
6. **White side test:** One ml of cervico-vaginal mucus was mixed with equal volume of 5% Sodium hydroxide (NAOH) solution and heated upto the boiling point in a water bath for two minutes as described by Pateria and Rawal (1990) [10].

Defense cell profile of uterine lavage
The uterine body was flushed by infusing 20 ml of phosphate buffer saline (PBS) or normal saline (NS) into the uterine body with a 60 ml sterile syringe attached to 52 cm sterile disposable plastic infusion sheath. The uterus was massaged and then retracted to recover the fluid. Fluid was recovered by negative pressure aspiration into the syringe and transferred to a 50 ml polystyrene centrifuge tube without any preservatives. Uterine lavage sample was brought to the laboratory within 2 hrs by placing it on ice in a small carrier. The fluid was centrifuged @1000 rpm for 10 minutes and the sediments were smeared on a glass slide, dried in air, fixed with methyl alcohol for 3 minutes and then smear was stained with Giemsa stain. A total of 100 cells were counted at 100X in each specimen and they were classified into PMNs, eosinophils, basophils, lymphocytes and monocytes.
The Total Leukocyte Count (TLC) of the uterine lavage was estimated by counting the leukocytes in all four primary squares of the haemocytometer. Leukocytes from the 4 WBC chamber of Neubauer's slide were counted and multiplied with the correction factor 50 to get the TLC of uterine lavage.
The uterine lavage was carried out and cellular material was collected on two occasions in white side test positive buffaloes of I, II (before and after treatment) and in control groups.

Study model
The study was carried out in 24 repeat breeder buffaloes. These buffaloes were categorized into treatment (n=14) and control (n=10) groups.

Table 1: Group wise treatment schedule of repeat breeder buffaloes

Sl. No.	Groups	Treatment	Dose regimen
1.	I (n=7)	Exapar-N®* + Janova®* +Mintrus®*	Day:0-5= Oral administration of Exapar-N® @100ml bid on first day followed by @50 ml bid Day:6-7= Capsule Janova®* 3 cap. /day for 2 days. Repeated the dose after 10 days. Day:0-20= Tablet Mintrus®* 1/day daily oral administration
2.	II (n=7)	Exapar-N® + Janova® +Mintrus®	Day:0-5 = Oral administration of Exapar-N®@50ml bid on first day followed by @25 ml bid Day:6-7 = Capsule Janova® 3 cap. /day for 2 days. Repeated the dose after 10 days. Day: 0-20=Tablet Mintrus® 1/day daily oral administration
3.	Control V (n=10)	No treatment	-----No treatment-----

Table 2: Ingredients of Exapar-N®

Sl. No.	Contents
1	*Plumbago zeylanica*
2	*Lepidium sativum*
3	*Citrullus colocynthys*

Table 3: Ingredients of Janova®

Sl. No.	Contents	Quantity in mg/capsule
1	Indravaruni	500
2	Pippali	50
3	Marica	50
4	Sunthi	50

Table 4: Ingredients of Mintrus®

Sl. No.	Contents	Quantity in mg/tablet
1	Iron	40
2	Manganese	50
3	Selenium	2
4	Zinc	45
5	Cobalt	8
6	Copper	100
7	Iodine	2

Follow up
All the buffaloes were inseminated in the subsequent 1st, 2nd and 3rd estrus periods if repeated after treatment. The pregnancy was diagnosed per rectally after 60 days of A.I.

Statistical analyses
The data obtained was subjected for statistical analyses as per methods described by Snedecor and Cochran (1980). Statistical analysis of experimental data was carried out by employing paired 't' test and ANOVA, using the "SAS 9.3" software which was procured from Indian Council of Agriculture Research under the NAIP project "Strengthening statistical computing for NARS".

Results and Discussion
Vaginal electrical resistance (VER): The mean value of

VER in infectious repeat breeder buffaloes was 262.50 ± 6.7. The mean value of VER in pregnant repeat breeder buffaloes was 242 ± 6.27 and in non-pregnant repeat breeder buffaloes was 262.04 ± 7.07. The difference between the VER values of pregnant and non-pregnant animals was statistically significant (P<0.05). The conception rate is more when VER values are less on the day of estrus in repeat breeder buffaloes. Sharma *et al.* (2004) [12] found significant association (P<0.01) between VER and non-return rates as well as with pregnancy rate (P<0.05). This result is in agreement with the findings of Meena *et al.* (2001) [13].

Physical characteristics of estrual mucus

1. **Color:** Infectious repeat breeder buffaloes exhibited 60.00% whitish, 40.00% translucent and 0.00% transparent color. Mehta (1986) [14] reported that, 54.17% of repeat breeder animals had clean and transparent cervical mucus. Samad *et al.* (2002) [15] reported that, estrus mucus was transparent in 55.00%, translucent in 38.33% and whitish in 6.67% repeat breeder buffaloes. Enkhia and Kohli (1982) [16] found 50.00% transparent, 30.00% translucent and 20.00% yellowish in repeat breeder cows.
2. **Consistency:** The estrual mucus consistency was thick in 60%, viscous in 40% infectious buffaloes. Higher pregnancy rate of 59.09% was recorded in buffaloes having viscous type of estrual mucus discharge followed by thick (27.72%) then followed by 18.18% in thin mucus discharge. These results are in agreement with Vadodaria and Prabhu (1990) [17]. In contrast, Sharma *et al.* (2011) [18] recorded 44.44% in viscous, 33.33% in thick and 22.33% conception rate in thin vaginal mucus. Deo and Roy (1971) [17] reported 59.10% and 32.90% of conception rates in buffaloes with thin and thick cervical mucus respectively. Gebhard and Schumacher (1970) [20] reported that, profuse, watery and clear cervical mucus was favourable for sperm penetration and thick, scanty and opaque cervical mucus was unfavourable for sperm penetration.
3. **pH:** The mean value of pH was 8.45 ± 0.10 in infectious repeat breeder buffaloes. The pH values are towards alkaline side. This is in agreement with Vadodaria and Prabhu (1990) [17], Salphale *et al*. (1993) [21], Samad *et al.* (2002) [15], Kumar *et al.* (2011) [22] and Ramsingh *et al.* (2013) [23] but Tsiligianni *et al.* (2001) [24] reported slightly acidic i.e. 6.5-6.7. Out of all repeat breeder buffaloes 72.72% conceived when the estrual mucus pH ranged from 7 to 8 followed by 13.63% conception rate in pH of both 7 and 8-9.
4. **Spinnbarkeit value:** The mean spinnbarkeit value of non-infectious repeat breeder buffaloes was 6.75 ± 0.81. Sharma *et al.* (2011) [18] reported average spinnbarkeit value of mucus was 9.35 ± 0.66 cm which is in close conformity with Rangnekar *et al.* (2002) [25]. Dodamani (2000) [26] reported higher spinnbarkeit value (24.67 ± 1.32) in repeat breeding cows this value was in conformity of with Mohanty *et al.* (1996) [27] and Chouduri *et al.* (1997) [28]. Among all experimental repeat breeder buffaloes, 54.54% conceived when the spinnbarkeit value was more than 10 cm followed by 27.27% conception rate in spinnbarkeit value ranged from 6-10 cm and 18.18% conception rate in spinnbarkeit value from 1-5 cm respectively. Patil (1987) [29] and Sharma *et al.* (2011) [18] reported that, buffaloes that conceived had significantly higher (P<0.01) mean spinnbarkeit value (12.94 ± 0.81 vs 7.91 ± 0.96 cm) as compared to those that did not conceive. But Jadhav (1996) [30] and Bennur (2004) [31] did not find such variation in fertile and infertile estruses and conceiving and non-conceiving cows (7.38 ± 5.60 and 8.05 ± 1.33 cm).
5. **Arborization pattern:** Out of all experimental buffaloes, 45.00% typical, 55.00% atypical and 00.00% nil arborization pattern was observed. Luktuke and Roy (1967) [32] reported the cervical mucus fern pattern as typical (72.7%), atypical (18.2%) and nil type (9.1%). Pandya *et al.* (1987) [33] reported 35.71% typical, 44.24% atypical and 19.05% nil type of fern pattern in buffaloes. Kumaresan *et al.* (2001) [34] reported that, out of 69 buffaloes, 40, 16 and 13 showed typical, atypical and nil fern pattern. Conception rate of 57.5% and 18.57% were obtained in buffaloes with typical and atypical fern pattern. Samad *et al.* (2002) [15] indicated that, fern pattern was typical in 55% atypical in 45.0% and 0% nil in repeat breeder buffaloes. Sharma *et al.* (2011) [18] reported typical, atypical and nil fern pattern of mucus was observed in 39.34%, 42.63% and 18.03% buffaloes.

It is concluded that higher conception rate in repeat breeder buffaloes was noticed when cervico-vaginal mucus was transparent, viscous with typical fern pattern, spinnbarkeit value more than 10 cm and with pH range from 7-8.

Defence cell profile of uterine lavage

The total leukocyte count (TLC) in uterine lavage of repeat breeder buffaloes was significantly higher 635.7 1±131.70 in Group I and 897.14±131.12 in group II before treatment and the TLC values were 228.57±56.54 and 328.57±75.48 after treatment respectively. In contrast, TLC was lower 941.66±190.35 initially and it increased to 1058.33±180 in control group. It indicates that, the Exapar-N®, Janova® and Mintrus® oral treatment may be effective in reducing uterine infection. The difference between the means of TLC before and after treatment within the groups was significant (P<0.05) in group I and II. However, there was no significant (P>0.05) difference in control group. Solmon *et al.* (2006) [35] found 00.09 ± 0.01 millions/ml total leukocyte count (TLC) in endometritis affected buffaloes which is in agreement with present study value. Pramod *et al.* (2002) [36] reported infected uteri had shown significant rise (10.89 ± 1.79 million/ml) in overall total nucleated and epithelial cell counts. Khaja (2010) [37] reported higher total leukocyte count in repeat breeding cows. These values are higher compared to the present study TLC values. A primary defense mechanism of the bovine uterus is phagocytosis of contaminating organisms by neutrophils in the uterine lumen. These cells are recruited from the circulation whenever antigenic material is present (Tizard, 1996) [38]. Migration of cells into the uterus is earliest mode of non-specific or cellular defense against microbial invasion but the type and number of cells present in the uterus depends on physio-pathological condition of reproductive tract (Hussain and Daniel, 1992) [39]. An elevated number of PMN cells reflect mild local inflammation. In clinical cases of metritis or endometritis, the influx of neutrophils into the uterine lumen is caused by the presence of bacteria or their endotoxins (Baranski *et al.*, 2012) [40]. Increased PMNs can reduce fertility through their effects on sperm function and viability, embryo survival and implantation (Butt *et al.*, 1991)

[41]. In animals without apparent signs of clinical endometritis, sub-clinical disease was diagnosed by measuring the proportion of neutrophils present in the sample collected by low volume lavage (Gilbert, 2004; Kasimanickam *et al*., 2004 and Kasimanickam *et al*., 2005) [42, 43, 44].

Table 5: Comparison of mean values of Neutrophil within the group

Group	N% – Before treatment	N% – After treatment	|t|	Remarks
I (Exapar-N® 100 ml)	57.42±6.89	21.42±5.26	11.97	Significant
II (Exapar-N® 50 ml)	50.85±6.74	22.14±2.89	5.74	Significant
(Control)	57.16±6.95	61.16±7.02	0.96	Non-significant

Plate 1: Estrus Detector showing vaginal electrical resistance value in a repeat breeder buffalo

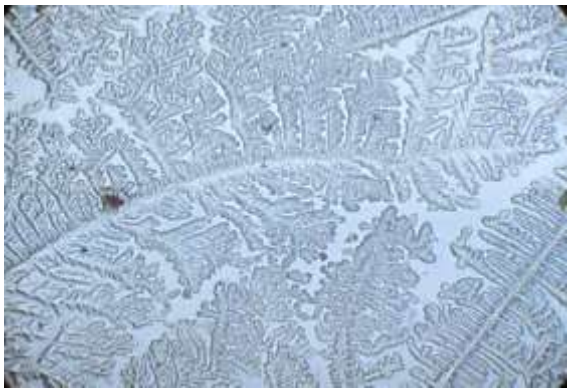

Plate 2: Typical fern tree leaves pattern in estrual mucus

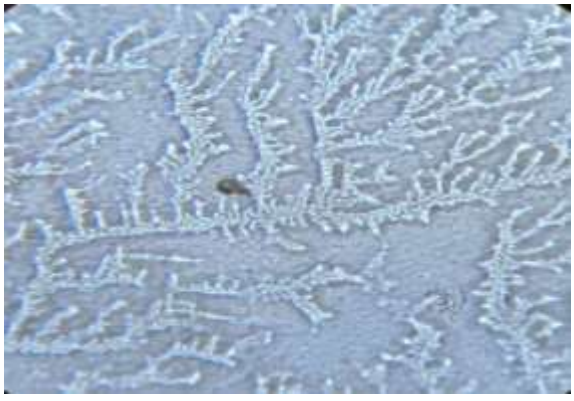

Plate 3: Atypical fern tree leaves pattern in estrual mucus

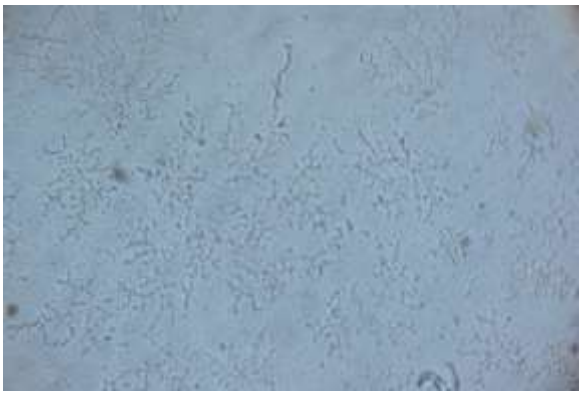

Plate 4: Nil fern tree leaves pattern in estrual mucus

Plate 5: Bubaline white side test for diagnosis of sub-clinical metritis {from left to right}: mild (light yellow), moderate (yellow), severe (deep yellow) and negative (no colour change)

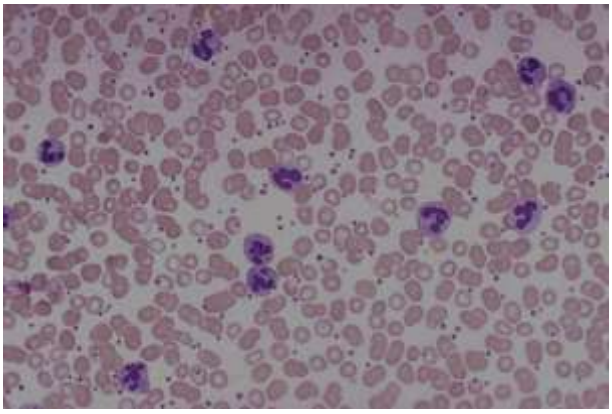

Plate 6: Increased number of neutrophils in uterine lavage before treatment in repeat breeder buffaloes

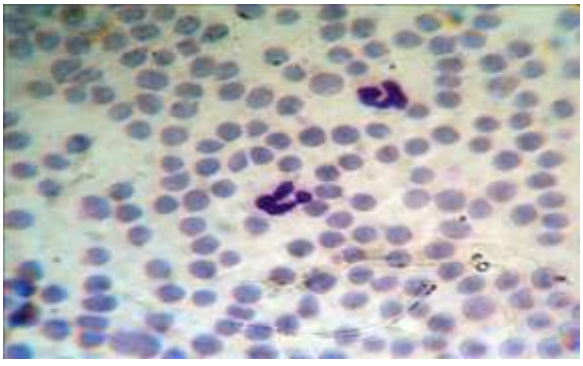

Plate 7: Decreased number of neutrophils in uterine lavage after treatment in repeat breeder buffaloes

The significant ($p<0.05$) difference was found within N% of groups I and II treatment while, no significant ($P>0.05$) difference was found within control group. Marked reduction in the PMN% was observed in both the treatment, it may be concluded that, both treatments of Exapar-N® 100 ml, Janova® and Mintrus® and Exapar-N® 50 ml, Janova® and Mintrus® oral treatment are efficacious in reducing the uterine infection. Klucinski *et al.* (1990) [45] revealed presence of an increased percentage of polymorphonuclear (PMN) cells up to 90% were noticed in the uterus during sub-clinical and clinical inflammation and once the offending contaminations were removed the inflammation subsided and the neutrophils no longer migrated to the uterus, therefore, the normal uterus examination for its luminal content revealed no neutrophils. Solmon *et al.* (2006) [35] recorded the cellular composition of uterine flushings and bacterial load of cervical mucus were more in endometritis affected buffaloes than healthy buffaloes. Azawi *et al.* (2008) [46] reported that, the mean neutrophils count were 41.1 ± 11.97% in repeat breeding buffaloes and 14.0 ± 2.02% in normal buffaloes which is in conformity with Ramesh *et al.* (2011) [47] and present study. Ayen *et al.* (2012) [48] reported that, maximum percentage of different defense cells distribution during luteal and follicular phases belonged to neutrophils (18.83 ± 3.23 and 14.80 ± 3.14, respectively) and the minimum percentage belonged to basophils (0.03 ± 0.02 and 0.00 ± 0.00, respectively). Cytology of uterine lavage revealed that, herbal uterine cleanser and restorative preparation is efficacious in reducing the uterine infection thereby increasing the conception rate.

It may be concluded that, cytological studies by uterine lavage can be used as an adjunct to diagnose sub- clinical uterine infections in buffaloes.

Efficacy of herbal uterine cleanser and restorative preparation

Out of 7 repeat breeder buffaloes 57.14% conceived with 2.43 services per conception in group I while, 14.28% conceived with 3 services per conception in group II. Paradeshi *et al.* (2009) [57] recorded the higher conception rate of 83.33%, 66.67%, 50%, 50% and 16.67% respectively in groups IV, III, II, I and V. All the Exapar® treated buffaloes became pregnant (100%), while only 50% of the animals became pregnant in the control group. For the Exapar® treated group, average number of services required per conception was just 1.75 as compared to 4 for the control group (Khanna *et al*., 1997) [49]. Namrata *et al.* (1998) [50] reported the average number of inseminations for conception and conception rate were better in the Exapar® treated buffaloes than control and observations were also in accordance with Singal (1996) [51], Srivastava (1997) [52], Namrata *et al.* (1998) [50], Dhakal (1999) [53], Gautam *et al.* (2005) [54]. Sahatpure *et al.* (2012) [55] reported administration of Exapar-N® is helpful in prevention of post partum reproductive disorders in cattle in reducing the number of AI and for improving conception rate. Koppad *et al.* (2009) [56] reported that, the administration of Exapar® liquid significantly improved the local immune response in animals as evident by a significant increase in total immunoglobulins, phagocytic response and polymorphonuclear cells in treated animals.

The present study regarding clinical efficacy of Exapar-N® at different dosage schedule revealed better conception rate with increase in the dosage which is in conformity with Paradeshi *et al.* (2009) [57].

Cytology of uterine lavage revealed that, herbal uterine cleanser and restorative preparation is efficacious in reducing the uterine infection thereby increasing the conception rate. The herbal uterine cleanser and restorative is efficacious in management of repeat breeding in buffaloes mainly due to sub-clinical endometritis and was satisfactory in terms of decreasing the load of PMN cells in the reproductive tract.

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