

Biochemical Changes in the Uterine Flushings of Repeat-Breeder Buffaloes

M.Z. Abd El-Aziz, Samira A. Emara and M.N.S.Sackran

*Pathology Dept., Biology Dept. and Field Investigation Dept. Animal
Reproduction Research Institute (ARRI) , Giza , Egypt .*

THIS STUDY was conducted to determine some biochemical alterations of uterine flushings in relation to uterine condition. For this reason, uterine flushings from 50 repeat-breeder buffalo-cows and 10 apparently normal buffalo-cows (control) were collected via Foley's balloon catheter using 40 ml sterile normal saline. Total protein concentration (T.P.), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine amino transferase (ALT) activities of the collected uterine flushings were determined. The mean values of T.P., ALP, LDH, AST and ALT of uterine flushings collected from repeat-breeder buffalo-cows were significantly higher than those of apparently normal buffalo-cows. This study indicates that the biochemical alterations (total protein and enzymatic activities) of uterine flushings can be used for diagnosis of endometritis along with microbiological isolations.

The buffaloes are the most important multi-purpose farm animals in the subtropical and tropical countries. The infertility in this species leads to great losses in the economy of developing countries (Shalash, 1984). The repeat-breeding is of the most important cause of infertility. Failure of conception may be due to several factors, among which endometritis is the most common one (Laing, 1970; Murthy *et al.*, 1974 and Hussain and Daniel, 1991).

In recent years, there has been an increasing interest in defining the biochemical nature of the intra luminal environment of the bovine female genital tract. The luminal fluid constitutes the environment in which

spermatozoa, ova and zygotes survive and function, that the constituents of these fluids influence early reproductive events as shown in many investigations (Malayer *et al.*, 1988 and Pong *et al.*, 1992).

There are different methods for the diagnosis of reproductive disorders, of which the laboratory examination of uterine flushing is a relatively easy procedure for the practicing veterinarian. Its use in conjunction with a detailed history could lead to a more accurate diagnosis and greater efficiency by the clinician.

There is less information on the biochemical changes in uterine flushings of repeat-breeding buffaloes, and most of the reports were based on the histopathological and bacteriological examinations. Therefore the present work was carried out to investigate some biochemical alterations in the uterine flushings, collected from repeat-breeder buffaloes, which can be used for screening the endometritis cases.

Material and Method

A total of 50 uterine flushings were collected from 50 repeat-breeder buffalo-cows presented for gynaecological examination to the artificial insemination center located at Shubramant village, Giza Province. All animals were free from congenital and anatomical defects of the genital tract. They were 4-9 years old with normal cycle and had been bred for more than 2-3 times to a fertile bull, but failed to conceive. The flushing was collected from each animal by infusing 40ml of sterile normal saline into the uterus through Folley's balloon catheter, then collected in a sterile container by gently massaging the uterus. The flushings were centrifuged at 3000 rpm for 15 minutes to remove the cellular debris and the supernatant fluid was transferred for storage at -20°C until analysis.

A total of 10 uterine flushings were collected from 10 healthy buffalo-cows which served as control.

The biochemical analysis of uterine flushing parameters included: Total protein concentration which was assayed by modified Biuret and Doumas method (Doumas, 1971). The enzymatic activities of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined spectrophotometrically by using test kits obtained from BioMereux, France according to Reitman and Frankel (1957) for ALT and AST, Belfield and Goldberg (1971) for ALP and McQueen (1972) for LDH respectively.

The data were statistically analysed using Student 't' test according to Snedecor and Cochran (1982).

Results

The mean total protein concentration in uterine flushings of repeat-breeder buffalo-cows was found to be significantly higher (0.568 ± 0.031 g%) in comparison to that of apparently normal cyclic buffalo-cows (0.430 ± 0.03 g%) (Table 1 and Fig. 1)

The average values of enzymatic activities in uterine flushings collected from repeat-breeder cases are presented in (Table 2 and Fig.2). The mean AST and ALT activities in uterine flushings of repeat-breeder cases were recorded to be highly significantly elevated (49.34 ± 6.78 and 31.62 ± 4.54 u/ml) when compared to those of apparently normal cyclic buffalo-cows (27.92 ± 3.21 and 14.04 ± 2.82 u/ml) respectively.

ALP and LDH activities in uterine flushings of repeat-breeder buffalo-cows were also higher (95.44 ± 5.88 and 338.5 ± 20.25 U/L) as compared to that of apparently normal cyclic buffalo-cows (77.30 ± 4.73 and 280.0 ± 18.56 u/L) respectively (Table 2 and Fig. 2).

Discussion

The total protein concentration in uterine flushings collected from repeat-breeder buffalo-cows were found to be significantly higher than that of healthy cases. This finding was in agreement with the observation of Williamson *et al.* (1983), who reported elevated level of protein concentration in uterine flushings collected from mares affected with endometritis. Furthermore, Rao and Seshagiri (1998) recorded higher level of protein concentration in uterine flushings collected from cows affected with endometritis in comparison to normal cows. The elevated level of protein concentration in uterine flushings of repeat-breeder buffalo-cows in this study might be due to increased level of secretory proteins and tissue damage which took place in the course of endometritis.

TABLE 1. Total protein concentration in uterine flushings of repeat-breeder cases and apparently normal cyclic cases.

Parameters	Apparently normal cyclic cases (n=10)	Repeat-breeder (n=50)
Total protein (g%)	0.430 ± 0.03	0.568 ± 0.03**

± Standard Error

** significant at p<0.01

TABLE 2. Enzymatic activities in uterine flushings of repeat-breeder cases and apparently normal cyclic cases.

Parameters	Apparently normal cyclic cases (n=10)	Repeat-breeder (n=50)
AST (U/ml)	27.92 ± 3.21	49.34 ± 6.78**
ALT (U/ml)	14.04 ± 2.82	31.62 ± 4.54**
ALP (U/L)	77.30 ± 4.73	95.44 ± 5.88*
LDH (U/L)	280.0 ± 18.56	338.5 ± 20.25*

± Standard error

* significant at p<0.05

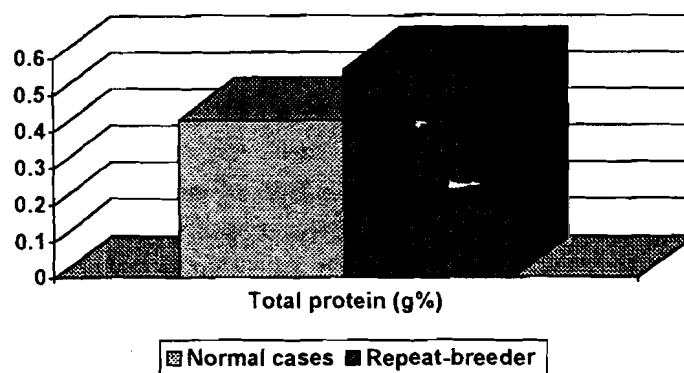
** significant at p<0.01

AST= Aspartate amino Transferase.

ALT= Alanine amino Transferase.

ALP = Alkaline Phosphatase.

LDH= Lactate Dehydrogenase.

**Fig . 1. Total protein concentration in uterine flushings of repeat-breeder cases and apparently normal cyclic cases.**

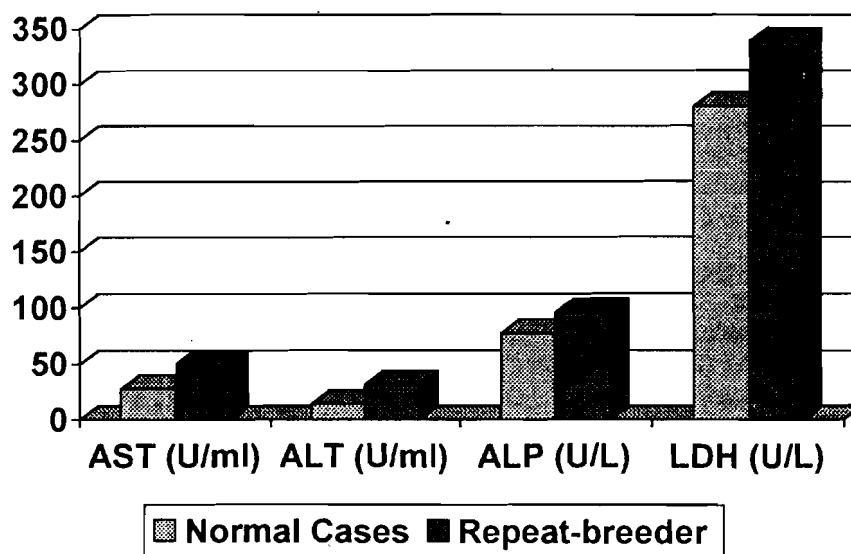


Fig.2. Enzymatic activities in uterine flushings of repeat-breeder cases and apparently normal cyclic cases.

The good knowledge of the enzymatic activity of bovine female reproductive tract under physiological and pathological conditions are important for diagnosis of infertility problems and improving reproductive efficiency (Zain El-Din *et al.* 1996). Serum, uterine fluid and tissue alkaline phosphatase activity during different reproductive stages in bovine have been studied by Peter *et al.* (1987), Boos *et al.* (1988), Zain El-Din *et al.* (1995) and Rao and Seshagiri (1998). Very little investigations have been reported on ALP activities in relation to uterine condition in non-pregnant cow (Boos *et al.*, 1988). According to the available data, there are no previous reports on ALP activity of uterine fluid from repeat-breeder buffalo-cows. Results of the present study showed highly significant elevation of ALP activity in uterine flushing from repeat-breeder cases than that of normal cases. This is in agreement with Boos *et al.* (1988), Zain El-Din *et al.* (1996) and Rao and Seshagiri (1998), who reported increased level of alkaline phosphatase activity in uterine flushings of cows and buffaloes affected with endometritis. The elevated level of ALP activity in repeat-breeder

cases may be due to cellular damage which took place as a result of inflammatory process and subsequent release of cellular content into uterine lumen.

Transaminases (AST and ALT) are present in small quantities in the serum of all animals as a consequence of normal tissue destruction and subsequent enzyme release (Coles, 1985). In the present study, the AST and ALT activities were higher in uterine flushings of repeat-breeder cases than that of the apparently normal cyclic cases. These were in agreement with records of Boos *et al.* (1988) and Zain El-Din *et al.* (1996).

Lactate dehydrogenase is an intracellular enzyme and widely distributed in animal tissues. The enzyme released following cellular damage (Coles, 1985). In the present study, LDH activity was higher in uterine flushings of repeat-breeder cases as compared with the normal cases. This is in agreement with earlier reports by Sommer and Marx (1969) and Zain El-Din *et al.* (1996) who found elevated levels of LDH in cows and buffaloes with clinical endometritis.

In conclusion, the results of this study suggest that the increased levels of total protein and enzymatic activities (ALP, AST, ALT and LDH) in uterine flushings were as a result of leakage of these substances from necrotic or damaged cells and can be used for diagnosis of endometritis in buffalo-cows, along with microbiological and histopathological examinations.

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التغيرات البيوكيميائية في الفسول الرحمي للجاوس المساب بالتبوع المتكرر

محمد زايد عبد العزيز، سميرة أحمد حمارة و محمد نجيب سعد
سکران

معهد بحوث التناسليات - الهرم - جيزة

أجرى هذا البحث لتقدير بعض التغيرات البيوكيميائية في الفسول الرحمي وعلاقتها بحالة الرحم. تم تجميع عدد 50 عينة فسول رحمي من حالات مصابة بالتبوع المتكرر (المجموعة المصابّة) ، وعدد 10 عينات فسول رحمي من حالات ذات دورة شبق طبيعية (المجموعة الضابطة) وذلك باستخدام 4 سم من محلول ملح فستولوجي معقم وقسطرة فولية، وتم تقدير مستوى البروتين الكلى - وخصائر الاستراتات أمينو ترانسفيراز - الالانين أمينو ترانسفيراز - الفوسفاتيز القاعدي وكذا خميرة اللاكتات ديهيدروجينيز في الفسول الرحمي .

أظهرت نتائج الفحص العملية للفسول الرحمي إلى وجود ارتفاع معنوي ملحوظ في قيم العناصر المذكورة في الحالات المصابة بالتبوع المتكرر عما هو في المجموعة الضابطة، وخلصت هذه الدراسة إلى امكانية استخدام التغيرات البيوكيميائية للبروتين الكلى والأنتريجات في الفسول الرحمي في المساعدة في تشخيص حالات الإلتهابات الرحمية جنباً إلى جنب مع الفزل الميكروبيولوجي.