Animal Reproduction Science 140 (2013) 40–46

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

# Association among energy status, subclinical endometritis postpartum and subsequent reproductive performance in Egyptian buffaloes

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ARTICLE INFO

*Article history:*
Received 25 January 2013
Received in revised form 10 May 2013
Accepted 12 May 2013
Available online 18 May 2013

*Keywords:*
Over all conception rate
Endometrial cytology
Ultrasonography
Buffalo cows
Blood metabolites
Postpartum

ABSTRACT

Sixty pluriparous, clinically normal buffalo cows ($n = 60$) were used to investigate the relationships between metabolic status, subclinical endometritis and reproductive performance. Subclinical endometritis was diagnosed by endometrial cytology (EC) and ultrasonography (US) during weeks 4–9 postpartum (pp). A comparative assessment of these diagnostic approaches was made with respect to reproductive outcomes. Blood samples were collected on a weekly basis from weeks 4 to 9 in order to estimate some blood metabolites including blood glucose, total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Reproductive tract examination was carried out weekly from weeks 4 to 9 by endometrial cytology (percentage of polymorphonuclear cells; PMN%) and Ultrasonography (detection of fluid in uterus regardless to its amount or echogenicity; FIU). The percentage of buffalo cow suffering from subclinical endometritis as diagnosed by endometrial cytology was significantly ($P < 0.01$) higher in non-pregnant cows (80%) at weeks 4 and 5 (60%) pp when compared with pregnant animals (0). HDL-c concentration was significantly lower ($P < 0.05$) in cytologically diagnosed ENDM group ($15.4 \pm 0.7$ mg/dl) if compared to NOENDM group ($25.0 \pm 3.1$ mg/dl) during week 4 pp. During week 5 pp, triglycerides concentration was significantly high ($P < 0.05$) in ENDM group, as diagnosed by the presence of FIU ($184.6 \pm 12.4$ mg/dl) if compared to NOENDM group ($102.7 \pm 30.6$ mg/dl). Total cholesterol concentration was significantly lower ($P < 0.01$) in ENDM group ($51.9 \pm 0.5$ mg/dl) than that of NOENDM group ($85.9 \pm 2.0$ mg/dl) during week 6 pp. In conclusion, Weeks 4 and 5 pp are the best times to identify cytologically diagnosed endometritis. Furthermore, glucose and total cholesterol indicated that energy status is not a risk factor for cytologically diagnosed subclinical endometritis in buffalo cows.



## 1. Introduction

Egyptian buffaloes are referred to as the Mediterranean buffalo of the river type. The buffalo, compared with all other domestic animals, produces high quality of milk characterized with 7% fat and more than 4% protein. The buffalo is a photoperiodic species and females show a decline in reproductive activity from mid-winter to spring in response to increasing day length (Zicarelli, 1997). The seasonal decline in reproductive activity is manifested by a reduced incidence of estrus behaviour, a decrease in the proportion of females that undergo regular estrous cycles and generally lower conception rates (Zicarelli, 1997).

Uterine function is often compromised in cattle by bacterial contamination of the uterine lumen after calving; pathogenic bacteria frequently persist, causing uterine

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0378-4320/$ – see front matter. 
http://dx.doi.org/10.1016/j.anireprosci.2013.05.004

inflammation, a main cause of infertility (Sheldon and Dobson, 2004). Uterine disease is associated with lower conception rate, increased intervals from calving to first service or conception, and more cattle culled for failure to conceive (Huszenicza et al., 1999). The major problems faced by buffalo breeders and farmers are poor reproductive efficiency and prolonged intercalving intervals (Jainudeen, 1986). Inflammation of the endometrium in the absence of clinical signs of endometritis is referred to subclinical endometritis (Sheldon et al., 2006). In cattle, diagnosis of subclinical endometritis can be carried out by uterine cytology or ultrasonography. For uterine cytology, samples are obtained by lavage technique (Gilbert et al., 2005; Barlund et al., 2008) or cytobrush method (Kasimanickam et al., 2004; Barlund et al., 2008; Senosy et al., 2009, 2011). Cytological smears are evaluated for their proportion of polymorphonuclear cells (PMN) by endometrial cells present in a sample. An increased proportion of PMN is prognostic for impaired subsequent reproductive performance (Kasimanickam et al., 2004; Gilbert et al., 2005; Barlund et al., 2008).

Reproductive performance of dairy cows after the voluntary waiting period is highly related to the health status of the uterus after calving (Plazier et al., 1997; Ferguson and Galligan, 2000). A complex relationship exists among factors influencing uterine health and disease in the postpartum cow (Gilbert, 1992; Kinsel, 1996). Energy status, particularly negative energy balance, has a major effect on reproductive perfprmance in buffaloes (Lubis and Fletcher, 1987; Wongsrikeao et al., 1990).

The objectives of the study were to validate the use of endometrial cytology (EC) and ultrasonography (US) to diagnose subclinical endometritis in clinically normal postpartum buffalo cows, to investigate the impact of metabolic status on subclinical endometritis, and a comparative assessment of these diagnostic approaches was made with respect to reproductive outcomes.

## 2. Materials and methods

### 2.1. Animals

Clinically normal pluriparous buffalo-cows ($n = 60$) with $507.14 \pm 18.9$ kg (mean ± SEM) live weight and body condition score (BCS) of $2.9 \pm 0.15$ were enrolled in the study during the period of September to December 2010. The cows were kept in free-stall barns and fed according to the NRC 2001. The ration (total mixed ration) included mainly alfalfa, corn silage, beet pulp, cottonseed, soya bean, corn, and barley. The buffalo cows were machine-milked twice daily. The mean 305-day milk yield of the cows in the preceding lactation was 2592.8 kg. Animals with abnormal vulvar discharges are excluded from the present study. Exclusion criteria were disorders including dystocia, retained placenta, caesarean section, mastitis, abnormal peripartum period and metabolic diseases such as clinical hypocalcaemia and ketosis (Younquist, 1997).

### 2.2. Study design

All buffalo-cows were examined six times on a weekly basis starting from 4-week pp. During the examination, cows were examined by transrectal ultrasonography (US) and endometrial brush cytology during weeks 4 ($25.5 \pm 2.8$ days in milk; DIM; mean ± SD), 5 ($32.4 \pm 2.1$ DIM), 6 ($41 \pm 2.9$ DIM), 7 ($49 \pm 2.4$ DIM), 8 ($55.7 \pm 3.2$ DIM) and 9 ($64.3 \pm 4.5$) after normal calving.

### 2.3. Endometrial cytology (EC)

Endometrial cytology was carried out according to Senosy et al. (2009). Endometrial cytology samples were collected by rotating the brush while in contact with the uterine wall (uterine body). The brush was retracted into the stainless steel tube prior to removal from the uterus. Cytology slides were prepared by rolling the brush on two clean glass microscope slides for each sample and fixed with cytofixative (Ahmadi et al., 2006).

Cytological evaluation (brush cytology) was used to estimate polymorphonuclear neutrophils percentage (PMN%) by counting a minimum of 200 cells of PMN and endometrial cells at 400× magnification for quantitative assessment of endometrial inflammation. Cows considered suffering from subclinical endometritis had PMN% ≥5% (ENDM group) while buffalo cows free from or containing PMN% <5% was considered subclinical endometritis free group (NOENDM group).

### 2.4. Transrectal palpation and ultrasonographic examination

Transrectal palpation was carried out to assess the position, uterine involution and the presence of fluid in the uterus regardless to its amount or echogenicity. Buffalo cows suffering from subclinical endometritis showed fluid in uterine body or uterine horns regardless of its amount or its nature (hyperechogenic or hypoechogenic) at the time of examination. Buffalo cows were considered free from subclinical endometritis when the uterus was free from any detectable fluid. An attempt to monitor uterine involution was done by measuring the weekly variation of the wall thickness of the uterine horns in a cross-sectional ultrasonographic image. Since it was difficult to achieve consistent measurements, uterine involution was considered complete when the uterus was decreased in size, easily retractable, had normal tonicity, completely located in the pelvic cavity and the difference in diameter between two uterine horns is ≤10 mm. Ultrasonographic evaluation was performed using a real-time, B-mode, diagnostic scanner equipped with a transrectal 6/8 MHz linear array transducer (Pie Medical, 100 LC, Holland).

### 2.5. Blood sampling, biochemical and steroids assays

Blood samples were collected from the jugular vein in vacuum tubes at weeks 4, 5, 6, 7, 8 and 9 postpartum. After centrifugation, plasma aliquots were frozen stored until metabolite and steroids determinations. Blood metabolites were analyzed by spectrophotometer (Unico, USA)