



# Mastitis outcomes on pre-ovulatory follicle diameter, estradiol concentrations, subsequent luteal profiles and conception rate in Buffaloes



Mohamed Mohsen Mansour<sup>a,\*</sup>, Moustafa M. Zeitoun<sup>b</sup>, Fekry M. Hussein<sup>c</sup>

<sup>a</sup> Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Damanhour, Al-Behirra, Egypt

<sup>b</sup> Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, Qassim University, Buriedah, Saudi Arabia

<sup>c</sup> Department of Theriogenology, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

## ARTICLE INFO

### Keywords:

Pre-ovulatory follicle  
Subclinical mastitis  
Clinical mastitis  
Luteal profile  
Conception rate  
Buffalo  
Estradiol

## ABSTRACT

The objectives of this study was to investigate the outcome of mastitis, in its clinical or subclinical forms, on the mean diameter of pre-ovulatory follicle (POF), plasma estradiol concentration on the day of estrus, subsequent luteal profile and subsequent conception rate in buffaloes. Sixty dairy buffalo (*Bubalus bubalus*) conducted in this study were divided into three groups {healthy (H),  $n = 20$ ; subclinical mastitis (SCM),  $n = 18$ ; and clinical mastitis (CM),  $n = 22$ }. Ultrasonography of ovaries revealed that mean diameter of POF was larger ( $P < 0.05$ ) in H buffalo (14.35 mm) compared to SCM (12.40 mm) and CM (10.25 mm). Also, plasma estradiol concentration on the day of estrus was higher ( $P < 0.05$ ) in H buffalo compared to SCM and CM counterparts; 34.95 vs. 32.87 and 27.50 pg/ml, respectively. Besides, positive correlation was observed between the POF diameter with plasma estradiol concentration in H, SCM and CM buffaloes ( $r = 0.64, 0.74, 0.72$  respectively,  $P < 0.05$ ). Moreover, positive correlations ( $P < 0.01$ ) were found on days 9, 12, 16, and 21 post-ovulation between POF diameter and luteal profile. Thus, the conception rate in H buffalo was higher ( $P < 0.05$ ) compared with SCM and CM counterparts; 55% vs. 38.89 and 18.18%, respectively. In conclusion, mastitis in its clinical or subclinical forms disrupts the functioning of the pre-ovulatory follicle on the day of estrus, associated with low follicular estradiol production, resulting in suppression to subsequent luteal profile leading to substantial decrease in pregnancy consequence of buffaloes.

## 1. Introduction

Buffaloes are the main dairy animals in some developing countries worldwide, supplying about 12% of world milk production. Despite this species tends to have relatively slow rate of reproduction and more reproductive problems such as inactive ovaries, long calving intervals and mastitis (Abd El-Razika et al., 2010). Mastitis is one of the most significant health problems of dairy herds as it cause physical, chemical and bacteriological changes in the milk of dairy buffaloes resulting in inferior quality and quantity of produced milk (Guccione et al., 2014, 2016a; Sharma et al., 2007).

Somatic cell count (SCC) is usually used as indicator of inflammation to diagnose mastitis (Dhakal et al., 1992; Singh and Ludri, 2001; Moroni et al., 2006). According to several studies in buffaloes, quarters producing milk with an SCC below the threshold of  $200 \times 10^3$  cells/mL and associated with negative bacteriological cultures (BC) are considered free from intramammary infections

\* Corresponding author.

E-mail address: [mohamedmohsen79@yahoo.com](mailto:mohamedmohsen79@yahoo.com) (M.M. Mansour).

(IMI) (Moroni et al., 2006; Guccione et al., 2014, 2016a; Tripaldi et al., 2010). Additionally, buffaloes producing milk with an SCC upon the threshold of  $200 \times 10^3$  and positive BC have been instead considered affected by IMI; are defined as suffering from subclinical mastitis (SCM), whereas in cases of clinical mastitis (CM), obvious signs of disease involving the appearance of the milk or udder are evident (Moroni et al., 2006; Guccione et al., 2014, 2016a; Tripaldi et al., 2010). Besides, clinical suspicion for the presence of mastitis is based on a combination of the following findings: visible changes of milk, atrophy or presence of indurations of the affected quarter (Radostits et al., 2007), presence of certain risk factors such as teat lesions or callused teats (Sieber and Farnsworth, 1984), positive result of the California Mastitis Test (CMT), and elevation of the SCC (Salvador et al., 2012; Schukken et al., 2003).

The economic impact of mastitis on reproduction failure has only gained attention in the last decade. However, almost data about the economic loss associated with reproduction failure, mentions to dairy cattle but little evidence is presented for buffalo's breeds. Although a high probability exists that these identified risk factors may also be observed among these species. Studies in dairy cattle have revealed associations between CM and increased odds of abortion (Risco et al., 1999), abnormal length interservice intervals (Moore et al., 1991), and failure to become pregnant after a service (Hertl et al., 2010). Other studies have identified associations between SCM and increased odds of embryonic loss (Moore et al., 2005), abortion, and failure to become pregnant to first service (Pinedo et al., 2009). Several potential mechanisms have been proposed to explain the effect of mastitis on reproductive performance. These are comprehensively reviewed by Hansen et al. (2004), but broadly encompass detrimental impact of inflammatory mediators on ovarian follicular function (Herath et al., 2007), intrauterine embryonic survival (Soto et al., 2003), decreased luteal-phase length (Hertl et al., 2010), and the balance of luteolytic versus luteotrophic prostaglandins after conception (Neuvians et al., 2004). Also, we have recently shown (Mansour et al., 2016) that incidence of mastitis revealed suppression to both corpus luteum (CL) diameter and function leading to significant reduction in probability of pregnancy rates in buffaloes.

Pre-ovulatory follicle (POF) is the prime structure behind the endocrinology of estrus in cattle and buffaloes, which produces substantial amounts of estradiol (Pandey et al., 2011; Perry et al., 2007; Rahman et al., 2012a, 2012b). It is speculated that POF diameter is important for the subsequent development of CL and, hence conception rate. A larger POF may generate a larger CL that will secrete more progesterone and hereby have a positive effect on pregnancy recognition and pregnancy rates (Binelli et al., 2009; Pandey et al., 2011). During the luteal phase CL diameters were larger in healthy buffaloes as compared with buffaloes having symptoms of SCM or CM (Mansour et al., 2016). Also, Low plasma progesterone concentration was shown in SCM and CM buffaloes compared to healthy buffaloes, resulting in a significant reduction in probability of pregnancy (Mansour et al., 2016). To our knowledge, no previous studies have investigated the impact of mastitis in its clinical or subclinical symptoms to POF diameter, steroidogenesis, subsequent luteal profile and conception rate in buffaloes. Therefore, the aims of the present study were to examine the outcome of mastitis in its clinical or subclinical forms on: (1) the diameter of POF, (2) plasma estradiol concentration, (3) correlation between the POF diameter with plasma estradiol concentration on the day of estrus and subsequent conception rate, and, (4) correlation between the POF diameter with subsequent luteal profile (CL diameter and plasma progesterone concentration).

## 2. Materials and methods

### 2.1. Animals enrolment

All the animals chosen in the present study were reared in the same breeding farm of approximately 300 dairy buffaloes, free from mandatory reportable diseases and located in El-Beheira governorate, north of Egypt. Sixty buffaloes (*Bubalus bubalus*) were selected for the present study by clinical examination that included (1) transrectal ultrasonography of ovaries for follicular development, (2) the presence of a corpus luteum to confirm cyclicity, (3) the absence of gross abnormalities of the reproductive tract, and (4) convenience milk sampling between one month before and one month after artificial insemination (AI). All animals were maintained in open yards that allowed 15 m<sup>2</sup> for each animal. Buffaloes were fed on diet that cover both maintenance and milk production requirements according to the recommendation of the National Research Council (NRC, 2001). Briefly, buffaloes were fed on green fodder (*Trifolium alexandrinum*) with an adequate amount of concentrate mixture (maize or wheat 60%, soybean 25%, wheat bran 10%, rice bran 5%, and common salt 1%). Mineral-balanced mixture blocks and clean drinking water were offered as free choice. Buffaloes were at postpartum (average  $181 \pm 21.65$  days), and parity (average  $3.75 \pm 0.62$ ) at the commencement of the experiment. Average body condition score of buffaloes according to a 5-point scoring method introduced by Ezenwa et al. (2009) and slightly modified by Guccione et al. (2016b) was  $3.3 \pm 0.5$ . Buffaloes were milked twice daily, and the average milk yield was  $12.65 \pm 2.87$  kg/day.

### 2.2. Milk sample and study design

Milk samples were collected twice per day for each animal immediately before regular morning and evening milking, as described by National Mastitis Council (2004). Duration of daily milk samples collection was one month before and one month after AI. On the days of sampling, each individual buffalo enrolled was individually submitted to a complete clinical examination of the udder health status. Local and systemic signs and changes in milk appearance were recorded during each sampling. Milk samples were created by mixing equal amounts of milk from all 4 quarters into a sterile test tube (BD Vacutainer, Oxford, UK). Samples were transported to the laboratory in cool containers until BC and SCC were performed. The SCC was determined by an automated fluorescent microscopic somatic cell counter (Bentley 150, Bentley Instruments, Inc., USA) as described by Locatelli et al. (2013), Mansour et al. (2016) and Moroni et al. (2006). The BC was performed according to guidelines of the National Mastitis Council (1999) as described by Guccione et al. (2014, 2016a). Briefly, 10  $\mu$ L of each milk sample was streaked on one quarter of a blood-agar plate (Merck KGaA, Darmstadt,

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