RETURN TO FERTILITY IN POSTPARTUM MEHSANA BUFFALOES AFTER THERAPEUTIC APPROACH FOR LARGE OVARIAN CYSTS AND INACTIVE OVARIES USING A SHORT-TERM PROGESTIN-BASED REGIME UNDER HIGLAND FIELD CONDITIONS IN THAILAND

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ABSTRACT

This study aimed to determine utility of a short-term controlled internal drug release (CIDR)-based protocol for hormonal therapy to large ovarian cysts and inactive ovaries in postpartum anestrous Mehsana buffaloes under field conditions in a highland area of Thailand. Anestrous buffaloes were diagnosed by transrectal ultrasonography as having ovarian cysts (n=5) or inactive ovaries (n=14). These 19 buffaloes were submitted to a CIDR-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-pregnant mare serum gonadotropin (PMSG)-based protocol with human chorionic gonadotropin (hCG) injections. Cows that exhibited estrus were bred with bulls. Pregnancy was diagnosed by transrectal ultrasonography at 35 days after mating; the non-pregnant cows were continuously resynchronized by the same protocol. In the initial synchronization and resynchronization phases, the estrous and pregnancy rates did not differ between buffaloes diagnosed with large ovarian cysts or inactive ovaries. Although the pregnancy outcome did not differ between the two groups, 57.9% of the anestrous Mehsana buffaloes became pregnant after therapy with the short-term CIDR-based protocol. Thus, these data demonstrated that a CIDR-$PGF_{2\alpha}$-PMSG-based protocol with hCG injection effectively treated large cystic and inactive ovaries in infertile buffaloes, allowing for subsequent pregnancy; this hormonal therapy is recommended for anestrous Mehsana buffaloes under field conditions in the highland areas of Thailand.

Keywords: Mehsana buffaloes, anestrus, highlands, inactive ovary, ovarian cyst

INTRODUCTION

Mehsana buffaloes (*Bubalus bubalis*) are distributed in Mehsana, Banaskantha and Sabarkantha districts of North Gujarat, India (Pundir *et al*., 2000; Muhaghegh and Goswami, 2006). The buffaloes are used for milk production. In 1996, the National Dairy Development Board of India provided Mehsana buffalo to the Royal Project Foundation of Thailand; these buffalo are reared in the highlands of northern Thailand. However, a majority of all cases of reproductive failures in postpartum Mehsana buffaloes result from infertility. In buffaloes, the delay in resumption of ovarian cyclic activity has been attributed to the long calving interval (Malik *et al*., 2011). In buffaloes, postpartum anestrus (inactive ovary) was reported to be as high as 31% to 42% for more than five months after calving (El-Wishy, 2007). Moreover, cystic ovaries have been recognised clinically as a cause of reproductive failures, and are an important

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ovarian dysfunction in buffaloes. The incidence of cystic ovarian disease has been reported to be 0.5% to 1.4% in buffaloes (Purohit, 2014). Due to these incidences, hormonal therapies are rarely utilised in buffalo reproductive management.

Until now, few studies have focused on a short-term progestin-based therapy for cystic and inactive ovaries in postpartum Mehsana buffaloes under field conditions in a highland area. We hypothesized that a therapeutic approach to treat large ovarian cysts and inactive ovaries with a short-term progestin-based protocol would influence the return to fertility and subsequent pregnancy outcome in postpartum Mehsana buffalo. Therefore, the objective of this present study was to determine utility of a short-term controlled internal drug release (CIDR)-based regime for hormonal therapy to large ovarian cysts and inactive ovaries in anestrous Mehsana buffaloes under field conditions in a highland area of Thailand.

MATERIALS AND METHODS

Experimental location, animal and feeding

This experiment was conducted at a buffalo dairy farm, the Mae Tha Nuea Royal Project Development Center, Thailand, situated at longitude 99°18′06.2″E, latitude 18°41′48.0″N and an altitude of 520 to 1,250 m above sea level (defined here as a highland area). The climate was tropical, with distinct differences between the dry (October to April) and wet (May to September) seasons. Nineteen postpartum anestrous Mehsana buffaloes (>90 days after calving) were treated for cystic and inactive ovaries. All buffaloes received chopped corn stover as roughage *ad libitum* and concentrate supplementation. Water was available *ad libitum*.

Ovarian diagnostic ultrasonography

Cysts and inactive ovaries were diagnosed by transrectal ultrasonographic genital monitoring with a 7.5 MHz rectal transducer (HS-1600 V, Honda Electronics, Japan). The buffalo cows were diagnosed with large ovarian cysts, if they had anovulatory follicle-like conformations (fluid-filled structures) larger than 25 mm in diameter on their ovaries (Noseir and Sosa, 2015) that persisted for more than 10 days without a corpus luteum (CL) (Purohit, 2014). The diagnostic criterion for inactive ovaries was the absence of luteal structures and follicles more than 10 mm in diameter (Yotov *et al*., 2012; Ingawale and Bakshi, 2016). Based on the transrectal ultrasounds of the ovarian structures, postpartum anestrous buffaloes were diagnosed with either cystic ovaries (n=5) or inactive ovaries (n=14).

Short-term CIDR-based protocol and pregnancy diagnosis

Nineteen Mehsana buffaloes, categorised into cystic and inactive ovarian groups, were submitted to a short-term CIDR-based protocol. Hormonal synchronization was adapted from previously described procedures (Yotov *et al*., 2012). Briefly, buffalo cows were treated with an intravaginal CIDR insert (Eazi-Breed CIDR, Zoetis Limited, Thailand) containing 1.9 g of progesterone (P_4) for 7 days. On the day of the CIDR withdrawal, 25 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Dinoprost, Lutalise, Zoetis Limited, Thailand) and 500 IU of pregnant mare serum gonadotropin (PMSG; Folligon, MSD-Animal Health, New Zealand) were injected intramuscularly. Two days after CIDR removal, buffalo cows received 500 IU of human chorionic gonadotropin (hCG; Chorulon, MSD-Animal Health, New Zealand). After hCG administration, all cows were observed for estrus with a teaser bull. Commencement of estrus was defined as the time when the cows displayed mounting behavior followed by vaginal mucus discharge, as well as enlarged vulva and vagina (Singh, 2003). Then, the cows that exhibited estrous behavior were bred with a Mehsana bull. Pregnancy was diagnosed by transrectal ultrasonography of uterine contents and fetal structures at 35 and 60 days after mating. Buffaloes diagnosed as not pregnant at day 35 were continuously resynchronized for a second round of hormonal therapy by a short-term CIDR-based protocol.

Statistical analysis

The proportion of buffalo cows in estrus and pregnant were analyzed using chi-square analysis (Steel *et al*., 1997) by procedure of SAS (SAS Institute Inc, Cary, NC, USA). Differences with $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

The incidence of infertility was observed, with the majority of the buffaloes having inactive ovaries (73.7%, Figure 1a) and some of them having large ovarian cysts (26.3%, Figure 1b). The present study provides the first description of the incidence of reproductive failures from inactive ovaries and large ovarian cysts in postpartum Mehsana buffaloes under field conditions in a highland area. This observation is inconsistent with Modi *et al*. (2011), who reported that the incidences of true anestrus and follicular cysts in dairy buffaloes in the Mehsana milk-shed area of Gujarat (India) were 20.84% and 0.07%, respectively.

In the initial synchronization and resynchronization phases, the estrous and pregnancy rates of buffaloes diagnosed with large ovarian cysts or inactive ovaries did not differ (Table 1). Overall, the pregnancy outcome after synchronized treatment did not differ significantly between inactive and cystic ovarian buffaloes (Table 1). Although the incidences of smooth inactive ovaries and large ovarian cysts in anestrous Mehsana buffaloes did not demonstrate a difference in the pregnancy outcome after therapy with the short-term CIDR-based protocol, more than 20.0% and 33.3% of the non-pregnant buffaloes in the two groups were diagnosed with now active ovaries, respectively (Table 1, Figures 1c and 1d). The estrous and pregnancy rates of the anestrous buffaloes did not differ significantly between the initial synchronization and resynchronization phases (Table 2).

These results demonstrated that treating postpartum anestrus Mehsana buffaloes with a hormonal protocol did not significantly different between those with cystic ovaries or inactive ovaries. The results of this study support the hypothesis that hormonal therapy for cystic ovarian and inactive ovarian buffaloes benefited the pregnancy outcome. More than 64.3% and 40.0% of the anestrous Mehsana buffaloes with inactive ovaries and large ovarian cysts, respectively, in the present study returned to pregnancy after initial synchronization and resynchronization using a short-term CIDR-based protocol. This was consistent with Yotov *et al*. (2012), who demonstrated that the pregnancy rate of buffalo diagnosed with inactive ovaries was 55.6% in the Murrah breed after therapy with a progesterone-releasing intravaginal device (PRID)-based protocol. Regardless of the cause of anestrous (cystic ovaries or inactive ovaries) in Mehsana

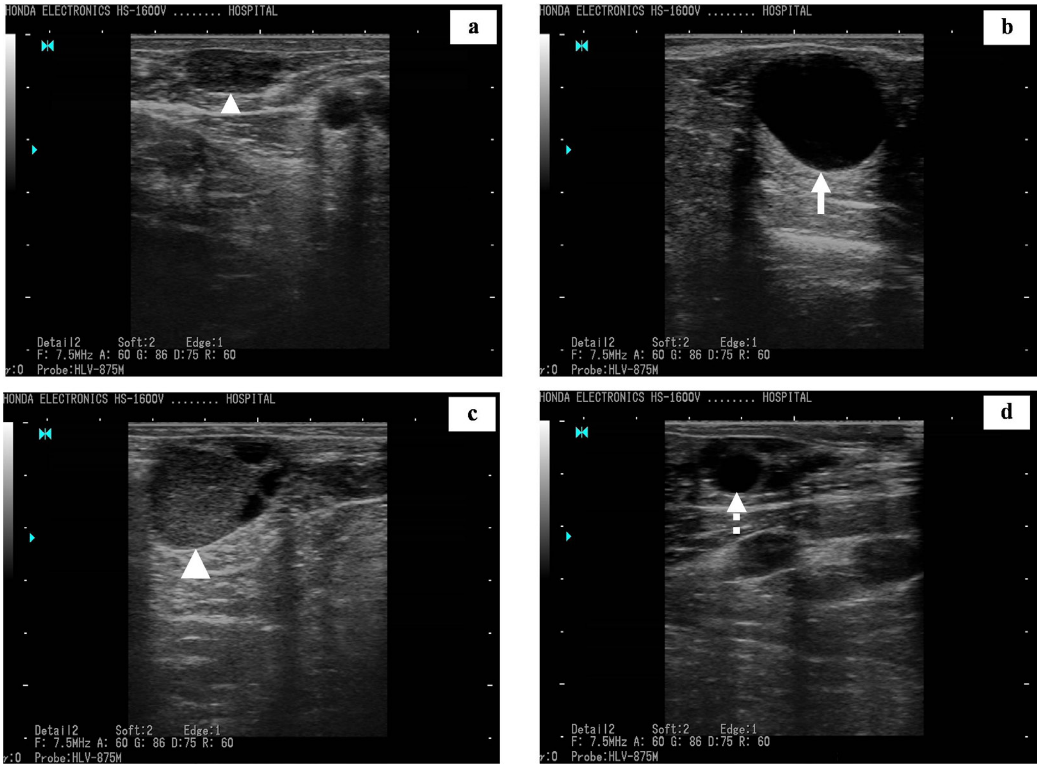

Figure 1. Sonogram images illustrate smooth inactive ovary (a: small arrowhead) and large ovarian cyst (b: arrow) of anestrous Mehsana buffaloes. After therapy with a short-term CIDR-based protocol, sonogram images illustrate non-pregnant buffaloes with active ovaries (c and d), as indicated by the appearance of a CL (large arrowhead) and/or follicles more than 10 mm in diameter (dotted arrows). The black areas in the sonogram images correspond to the follicle areas.

Table 1. Estrous and pregnancy responses of anestrous Mehsana buffaloes after therapy for large ovarian cysts and inactive ovaries with a short-term, CIDR-based regime.

Item	Buffalo group	
	Cystic ovary	Inactive ovary
Initial synchronization		
Buffalo cows (no.)	5	14
Estrous rate (%)	40.0 (2/5)	50.0 (7/14)
Pregnancy rate (%)	50.0 (1/2)	42.9 (3/7)
Resynchronization		
Buffalo cows (no.)	4	11
Estrous rate (%)	50.0 (2/4)	72.7 (8/11)
Pregnancy rate (%)	50.0 (1/2)	75.0 (6/8)
Pregnancy outcome (%)	40.0 (2/5)	64.3 (9/14)
Non-pregnant buffaloes with active ovaries (%)[a]	33.3 (1/3)	20.0 (1/5)

[a]The percentage of non-pregnant buffaloes with active ovaries was calculated per total buffalo cows for return to active ovaries, as indicated by the appearance of a CL and/or follicles more than 10 mm in diameter.

Table 2. Estrous and pregnancy responses of anestrous Mehsana buffaloes after initial synchronization and resynchronization with a short-term CIDR-based regime.

Item	Synchronization phase	
	Initial synchronization	Resynchronization
Buffalo cows (no.)	19	15
Estrous rate (%)	47.4 (9/19)	66.7 (10/15)
Pregnancy rate (%)	44.4 (4/9)	70.0 (7/10)
Pregnancy outcome (%)	21.1 (4/19)	46.7 (7/15)

buffaloes, a short-term CIDR-PGF$_{2\alpha}$-PMSG-based program with hCG injection influenced estrous activity and increased the pregnancy outcome by 57.9%. It has been suggested that a P$_4$-PGF$_{2\alpha}$-PMSG-based protocol for treating cystic ovaries and stimulating ovarian activity in buffalo cows (Yotov *et al*., 2012) activates the maturation of dominant follicles, either directly through the action of PMSG or indirectly by P$_4$ device synchronizing a surge of luteinizing hormone (LH) (Naseer *et al*., 2013). In fact, the successful therapy of ovarian cysts was improved 65% to 80% by stimulating the LH concentration with hCG. The hCG was used because of its greater LH activity (Noseir and Sosa, 2015). Moreover, P$_4$ device disintegrates cysts by resuming the responsiveness of the hypothalamus to the positive feedback mechanism of estradiol, which influences atresia of cysts, and subsequently retunes estrus and ovulation after P$_4$ device withdrawal (Jeengar *et al*., 2014).

Based on our results, 1) stimulating the endocrine milieu that enhanced the follicular growth by using PMSG, 2) inducing the atresia of cysts by using a CIDR device, and 3) influencing the ovulatory activity by using hCG improved the traditional therapeutic treatment approach for large ovarian cysts and inactive ovaries in anestrous buffaloes. Thus, a CIDR-PGF$_{2\alpha}$-PMSG-based protocol, in combination with hCG, effectively treated large cystic and inactive ovaries in infertile Mehsana buffaloes, returning the cows to fertility; this hormonal therapy is recommended for anestrous Mehsana buffaloes under field conditions in the highland areas of Thailand.

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