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Treatment of Anestrous in Iraqi Buffaloes using Ovsynch alone or in Combination with CIDR

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Abstract

The aim of this study was to test the efficacy of the Ovsynch alone or in combination with CIDR treated buffaloes on conception rate of anestrous Iraqi buffaloes. The present study was conducted on 50 anestrous buffaloes suffering from postpartum ovarian inactivity. Buffaloes were randomly assigned to three treatment groups. Buffaloes in Treatment 1 (n=20) received on day 0 GnRH 250 μg and a 25 mg of PGF2α on day 7, two days later a second injection of GnRH (250 μg). Buffaloes in Treatment 2 (n=20) injected on day 0 of the experiment, GnRH 250 μg and a CIDR was placed in the anterior vagina. On day 7, they were injected with 25 mg of PGF2α and on the following day (day 8) the CIDR were removed. On day 9 a second dose of GnRH (250 μg) was injected. Treatment 3 a control group (n=10) received no treatments. Results showed that in treatment 1, 7 out of 20 buffaloes received treatment exhibited estrus within 74.8 ± 6.3 after second GnRH administration. The estrus induction rate was 35%. Total number of buffaloes became pregnant was 1 (5%). In treatment 2 75% of the buffaloes received treatment exhibited estrus within 77.8 ± 5.6 h after CIDR removal. Total number of buffaloes became pregnant was 7 (31.8%). Lower (P <0.05) Pregnancy rate was achieved in buffaloes treated with Ovsynch than other buffaloes treated with Ovsynch plus CIDR. Higher rates of estrus induction (75%; $P<0.05$) and conception rate (7 buffaloes out of 20) were found in buffaloes treated with Ovsynch plus CIDR. In conclusion, our results indicate that the addition of Ovsynch to a progesterone-based CIDR protocol substantially improves the estrus induction and pregnancy rates in postpartum anestrous buffaloes.

*Keywords:*Buffalo; Anestrous; Ovsynch; CIDR; GnRH; PGF2α

Introduction

Anestrous is generally defined as the state of ovarian acyclicity, reflected by complete sexual inactivity without manifestation of estrus (Wright and Malmo, 1992). Postpartum anestrous is the period after parturition during which buffalo cow do not show behavioral signs of estrus. True anestrous condition is associated with the presence of static ovaries, and even though there is follicular development, none of the ovarian follicles that start growing becomes mature enough to ovulate. The period of postpartum anoestrus or anoestrus is usually longer in buffalo than in cattle under comparative management conditions (Jainudeen, 1988).

The Ovsynch protocol combines treatments with GnRH and prostaglandin F2α to synchronize the time of ovulation. Although much work has been done using ovsynch ovulation for reproductive management technique in cattle, especially in dairy cows (Pursley *et al*. 1995) and has already found application with equally encouraging results in buffaloes (Ahmed *et al.*, 2010). As for the use of prostaglandin, the efficacy of this protocol finds its limitation in that only cyclic animals can be used. In order to optimize treatment protocols and pregnancy rates in the buffalo species, it is mandatory to improve the degree of achieved synchronization between ovulation of dominant follicle and the fertilization, and to understand the fate of dominant follicle available at the beginning of the synchronization protocol. Such dominant follicles may become themselves ovulating follicles (no-follicle shift) or may regress giving way to a new dominant and ovulating follicle (follicle shift) at the end of the protocol. Incorporation of a controlled internal

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drug release (CIDR) insert containing progesterone from GnRH to PGF2α (Stevenson *et al*., 2006), prevented premature estrus and increased conception rates. Information on their use in buffalo's especially postpartum anestrous lactating Iraqi buffaloes has never been undertaken. Therefore, the present study was undertaken to test the efficacy of the Ovsynch alone or in combination with CIDR treated buffaloes on conception rate of anestrous Iraqi buffaloes.

Materials and methods

Animals

The study protocol was approved by the Ministry of Agriculture, The State Company for Animal Resources Services. The study began in April 2011 and continued until September 2011. This study was conducted on Iraqi northern buffalo cows in Nineveh province housed at smallholders' dairy farms (latitude: 36º 20´N, longitude: 43º 8´E). Postpartum lactating buffaloes included in this study had a mean age 9. 77 ± 0.62 y, mean body weight 361.8 ± 11.9 kg, mean body condition score 3.26 ± 0.02 (which is a subjective, visual assessment based on rib visibility, rump-fat thickness and fat thickness around the hooks and pins). The animals were kept outdoors near the rivers for wallowing and milked twice daily. A balanced nutritional diet including green fodder and concentrate mixture were fed to these animals. Animals with a history of caesarean operation, cystic ovaries, pyometra, metritis, endometritis, lameness, abdominal disorders or other undercurrent diseases were excluded from the study on the basis of clinical examinations to remove any conflicting influence during the study period. Following data was recorded for each buffalo cow included in the present study: name of buffalo, breed, number of parturitions, obstetrical problems if present, type of last parturition, retained placenta, vaginal prolapse, uterine prolapse, abortion, number of services, and milk production .

Clinical examination

Rectal examination of uterus, uterine tubes, ovaries and cervix was performed to each buffalo cow with smooth ovaries (without palpable corpus luteum and follicle). Any buffalo with abnormal vaginal discharge was excluded from the study. All buffalo cows were characterized with postpartum anestrous of ≥ 90 days of the last parturition without any signs of estrous or pregnancy. Rectal examination was performed secondly at 10 days interval to confirm examination of all buffaloes included in this study were having smooth inactive ovaries without palpable corpus luteum and follicle.

Treatment protocols

Buffaloes were randomly assigned to three treatment groups. Buffaloes in Treatment 1 (n=20) received on day 0 GnRH 250 μg (Cystorelin, Ceva Sante Animale, La Ballastiere-33501, Libourne Cedex, France) as the dose suggested for Iraqi buffaloes by Rahawy *et al.* (2006), and a 25 mg of PGF2α (Intervet, B.V., Boxmer, Holland) on day 7, two days later a second injection of GnRH (250 μg). Buffaloes in Treatment 2 (n=20) injected on day 0 of the experiment, GnRH 250 μg and a CIDR was placed in the anterior vagina. On day 7, they were injected with 25 mg of PGF2α and on the following day (day 8) the CIDR were removed. On day 9 a second dose of GnRH (250 μg) was injected. Treatment 3 a control group (n=10) received no treatments. To reduce the possibility of CIDR removal by pen mates or other buffaloes, CIDR tails were clipped to be appearing on vulva lips. Estrus was detected on farm by expert personnel using visual estrus detection every 3 hours (8 times daily). Due to the unavailability of artificial insemination of buffaloes in Iraq, all buffalo cows were mated naturally at least two times after estrous detection with buffalo bulls of proven fertility. Pregnancy status was diagnosed by palpation of uterine contents 35 days after the natural mating of the 1st induced estrous and the next 35 days of buffaloes not pregnant in 1st mating and mated in the 1st spontaneous estrous.

Results

Numbers and percentages of buffaloes exhibited estrus after Ovsynch and Ovsynch plus CIDR treatments and pregnancy results are shown in Table 1. In treatment 1(Ovsynch), 7 out of 20 buffaloes received treatment exhibited estrus within 74.8 ± 6.3 h after CIDR removal. The estrus induction rate was 35% for buffaloes treated with Ovsynch. Total number of buffaloes became pregnant was 1 (5%), 1 buffaloes conceived in the 1st induced estrus and

Table 1. Pregnancy rate of buffaloes with postpartum anestrous treated using Ovsynch alone or Ovsynch plus CIDR.

Treatment protocols	No. Animals	Buffaloes exhibited estrous		Buffaloes pregnant in 1st induced estrous		Buffaloes pregnant in 1st spontaneous estrous		Total pregnant	
		No.	%	No.	%	No.	%	No.	%
Treatment 1 (Ovsynch alone)	20	7	35b	1/7	14.3b	0	0	1/20	5b
Treatment 2 (Ovsynch + CIDR)	20	15	75a	4/15	26.6a	3/11	27.3b	7/20	31.8a
Treatment 3 (Control)	10	2	10b	0	0	0	0	0	0

a,b: Values with different superscripts within the same column are significantly different; P< 0.05.

no one conceived in the 1st spontaneous estrus. In treatment 2 (Ovsynch plus CIDR), 15 out of 20 buffaloes received treatment exhibited estrus within 77.8 ± 5.6 h after CIDR removal. The estrus induction rate was 75% for buffaloes treated with Ovsynch plus CIDR protocol. Total number of buffaloes became pregnant was 7 (31.8%), 4 buffaloes conceived in the 1st induced estrus and the remaining 3 conceived at the 1st spontaneous estrus. In treatment 3 none of the animals in the control group conceived. Lower (P <0.05) Pregnancy rate was achieved in buffaloes treated with Ovsynch than other buffaloes treated with Ovsynch plus CIDR. Higher rates of estrus induction (75%; P<0.05) and conception rate (7 buffaloes out of 20) were found in buffaloes treated with Ovsynch plus CIDR.

Discussion

Buffaloes are much known as “Peculiar Shy Breeder” (Suthar and Dhami, 2010). Buffaloes are considered with lower number of primordial follicles observed in the buffalo ovary, varying from 10,000 to 19,000 (Danell, 1987) compared with 150,000 in cattle (Erikson, 1966) and lower number of antral follicles throughout the whole estrous cycle with high incidence of follicular atresia (El-Wishy, 2007a). Buffaloes have long postpartum ovarian inactivity leading to poor fertility (El-Wishy, 2007b). Anestrous is the state of ovarian acyclicity, reflected by complete sexual inactivity without manifestation of estrus (Wright and Malmo, 1992), and anovulation accompanied by serum progesterone concentrations less than 0.5 ng/ml (Arreguin *et al*., 1997). The main factors affecting the duration of postpartum anestrous in buffaloes are the nutritional status (El-Wishy, 2007b) and suckling (Jainudeen, 1988). The possible mechanism by which under-nutrition may inhibit resumption of postpartum ovarian activity in cows is by the inhibition of hypothalamic GnRH secretion. However, little is known about the specific ways in which information about nutritional state is translated into neuro-endocrine signals that affect GnRH secretion (Jolly *et al*., 1995). McShane *et al.* (1993) has been suggested that central effects of under-nutrition on hypothalamic GnRH secretion may be partly mediated by the neuropeptide Y, which also appears to play an important role in the regulation of feed intake and energy balance in ruminants. Suckling is one of the main factors that influence the length of the postpartum anestrous period (Jainudeen, 1988). If suckling is continuously practiced during lactation, it blocks ovulation with the consequent long period of postpartum anestrous, contributing to a lesser reproductive efficiency. Suckling and milk yield can affect activity of the hypothalamus, hypophysis and ovaries, inhibiting follicular development and extending the anestrous period. The amount of milk produced during the postpartum period can affect the interval from calving to ovulation. Suckling reduces the hypothalamic release of GnRH, which results in insufficient pulsatile LH release. Inhibition of pulsatile LH secretion during lactation and the corresponding increase in pulsatile LH secretion 2–6 days after removal of the suckling stimulus is well established (Williams *et al*., 1987).

Pursley *et al*. (1995) demonstrated that a combined regimen of GnRH on day 0, PGF2α on day 7 and GnRH on day 9, followed by a single fixed-time insemination can result in normal fertility. The first injection of GnRH is given at a random stage of the cycle and causes either ovulation or luteinization of a dominant follicle, if present, in the majority of animals. The rationale for the second administration of GnRH 2 days after PGF2α is to advance the time of the LH surge and consequently advance and synchronize ovulation, so that a single insemination is sufficient to ensure normal fertility. This GnRH – PGF2α – GnRH protocol, commonly known as Ovsynch, synchronizes follicular development, luteal regression and time of ovulation, thus permitting timed AI after the second GnRH administration. CIDR inserted intra-vaginally which are saturated with progesterone causes an increased circulatory concentration of progesterone exerted negative feedback on hypothalamus and anterior pituitary. Hence, result in favoring GnRH, FSH and LH storage. Following termination of progesterone therapy (after CIDR withdrawal by the day 7 after insertion), the rapid drop in circulatory concentration of progesterone promotes the release of GnRH as the negative feedback of progesterone was abolished, followed by FSH an LH release with subsequent resumption of ovarian cyclicity. Also, the increased circulatory concentration of progesterone has sensitized the hypothalamic-pituitary system (Singh *et al*., 2003). Likewise, progesterone increased hypothalamus sensitivity to estrogen with subsequent increase in the intensity of heat (Fabre-Nys and Martin, 1991; El-Wishy, 2007b). The estrus induction rate using Ovsynch plus CIDR showed 75% animals in the treated group expressed estrus. This result is in agreement with Ahmed *et al*. (2010). The main action of GnRH used at the start of progesterone treatment is to synchronize emergence of a new cohort of follicles (Rhodes *et al*., 2003). Also, second injection of GnRH has the additional effect of inducing ovulation and the formation of a corpus luteum in a majority of cows, resulting in elevated concentrations of progesterone in anestrous buffaloes. GnRH synchronizes the development and occurrence of follicles and results in more homogenous follicular development. Also, it induces ovulation or luteinization of dominant follicle in non cyclic animals. However the induced ovulation in non cyclic animals stimulated luteal tissue development and function resulting in the occurrence of cyclic activity (Bao *et al*., 2003). This work has also shown that the use of CIDR together with GnRH and PGF2α treatment is able to induce fertile estrus in non-cycling postpartum buffaloes. This result is in agreement with the results obtained in beef cattle (Martinez *et al*., 2011), dairy cows (Thatcher *et al*., 2006). This has an economic impact on buffalo production as a greater proportion of non-cycling postpartum buffaloes can be bred early. In fact, the Ovsynch plus CIDR protocol treatment increased the proportion of buffalos that became cyclic within a few days from the start of the trial. Moreover, treated animals had a higher conception rate compared with buffaloes treated with Ovsynch alone and controls. Inserting CIDR at the initial GnRH injection of the Ovsynch program (Ovsynch plus CIDR) and then removing the CIDR at PGF2α has been demonstrated to improve pregnancy rates in lactating buffaloes (Ravikumar *et al*., 2011) especially buffaloes that are anestrous (Zaabel *et al*., 2009). Inclusion of a CIDR with the Ovsynch program is most beneficial when a presynchronization program is not implemented. Also, exogenous progestin treatment during the interval between GnRH and PGF2α injection prevented premature estrus and increased conception rates of anestrous animals. Anestrous buffaloes exhibiting cycles with two follicular waves (Azawi *et al*., 2009) and initiating ovsynch during the later stages of estrous cycle would exhibit the estrous and higher conception rates because of the greater likelihood of ovulation in response to the first GnRH injection which would prevent premature ovulation and asynchrony with mating. In conclusion, our results indicate that the addition of Ovsynch to a progesterone-based CIDR protocol substantially improves the estrus induction and pregnancy rates in postpartum anestrous buffaloes.

References

Ahmed, W. M., El-Khadrawy, H.H., Abd El Hameed, A.R., Amer, H.A., 2010. Applied investigations on ovarian inactivity in buffalo heifers. International Journal of Academic Research 2, 26-32.

Arreguin, J.A.A., Santos, R.E., Villa-Godoy, A., Román-Ponce, H., 1997. Dinámica folicular ovárica en vacas Cebú con diferente condición corporal y frecuencia de amamantamiento durante el per´ıodo anovulatorio posparto. División de Educación Continua, Unam, F.M.V.Z. (Eds.), VII Curso Internacional de Reproducción Bovina. Méx., D.F., pp. 210–240.

Azawi, O.I., Ali, A.J., Noaman, U.T., 2009. A study on the ovarian follicular dynamic in Iraqi Northern Buffaloes. Tropical Animal Health and Production 41, 79–83.

Bao, G.A., Baruselli, P.S., Marques, M.F., 2003. Pattern and manipulation of follicular development in bos indicus cattle. Animal Reproduction. Science 15, 307-326.

Danell, B., 1987. Estrous behavior, ovarian morphology and cyclical variation in follicular system and endocrine pattern in water buffalo heifers. PhD Thesis, Swedish University of Agricultural Sciences, Upssala, Sweden, pp. 112.

El-Wishy, A.B., 2007a. The postpartum buffalo II. Acyclicity and anestrus. Animal Reproduction Science 97, 216–236.

El-Wishy, A.B., 2007b. The postpartum buffalo I Endocrinological changes and uterine involution. Animal Reproduction Science 97, 201-215.

Erickson, B.H., 1966. Development and senescence of the postnatal bovine ovary. Journal of Animal Science 25, 800–805.

Fabre-Nys, C., Martin, G.B., 1991. Roles of progesterone and oestradiol in determining the temporal sequence and quantitative expression of sexual receptivity and preovulatory LH surge in the ewe. Endocrinology 130, 367-379.

Jainudeen, M.R., 1988. Reproduction problems of buffaloes in the world. In: Proceedings of the Second World Buffalo Congress, vol. II, New Delhi, India, pp. 189-196.

Jolly, P.D., McDougall, S., Fitzpatrick, L.A., Macmillan, K. L., Entwistle, K. W., 1995. Physiological effects of undernutrition on postpartum anoestrus in cows. J. Reproduction and Fertility Supplement 49, 477–492.

Martinez, M. F., Nava, G. de., Demmers, K. J., Tutt, D., Rodriguez Sabarros, M., Smaill, B., Corti, M., Juengel, J., 2011. Intravaginal progesterone devices in synchronization protocols for artificial insemination in beef heifers. Reproduction and Domestic Animal (In press).

McShane, T.M., Petersen, S.L., McCrone, S., Keisler, D.H., 1993. Influence of food restriction on neuropeptide-Y, pro-opiomelanocortin, and luteinizing hormone-releasing hormone gene expression in sheep hypothalami. Biology of Reproduction 49, 831–839.

Pursley, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2a and GnRH. Theriogenology 44, 915–923.

Rahawy, M.A., Taha, M.B., Azawi, O.I., 2006. Clinical study of anestrous in Iraqi buffaloes in Nineveh province. Iraqi Journal Veterinary Science 20, 113-124.

Rhodes, F.M., McDougall, S., Burke, C.R., Verkerk, G.A., Macmillan, K.L., 2003. Treatment of Cows with an Extended Postpartum Anestrous Interval. Journal of Dairy Science 86, 1876–1894.

Singh, S., Wani, N.A., Maurya, S.N., 2003. Use of different hormones for the treatment of postpartum anestrous in buffaloes under field condition. Indian Journal of Animal Science 73, 894-896.

Stevenson, J.S., Pursley, J.R., Garverick, H.A., Fricke, P. M., Kesler, D. M., Ottobre, J. S., Wiltbank, M. C., 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. Journal of Dairy Science 89, 2567–2578.

Suthar, V. S., Dhami, A. J., 2010. Estrus Detection Methods in Buffalo. Veterinary World 3, 94-96.

Thatcher, W.W., Bilby, T.R., Bartolome, J.A., Silvestre, F., Staples, C. R., Santos, J. E. P., 2006. Strategies for improving fertility in the modern dairy cow. Theriogenology 65, 30–44.

Williams, G.L., Koziorowski, J., Osborn, R.G., Kirsch, J. D., Slanger, W. D., 1987. The postweaning rise of tonic luteinizing hormone secretion in anestrous cows is not prevented by chronic milking or the physical presence of the calf. Biology of Reproduction 36, 1079–1084.

Wright, P.J., Malmo, J., 1992. Pharmacologic manipulation of fertility. Veterinary Clinic North America Food Animal Practice 8, 57–89.

Zaabel, S.M., Hegab, A.O., Montasser, A.E., El-Sheikh, H., 2009. Reproductive performance of anestrous buffaloes treated with CIDR. Animal Reproduction 6, 460-464.