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Efficacy of 'co–ins'–sync protocol on fertility of anestrus buffaloes

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ABSTRACT

Major production factor influencing buffaloes is their reproductive efficiency. Out of the various reproductive disorders observed in buffaloes, postpartum anestrum is the principal cause of economical loss to the farmers. The present study was designed to assess the effect of CO–sync and 'CO–INS'–sync protocol with fixed time insemination in post-partum anestrus buffaloes which had failed to conceive after progesterone therapy with CIDR. Apparently healthy graded true anestrous parous Murrah buffaloes (n=12) with 5–10 years of age which failed to get conceived after CIDR treatment (75–120 days) were selected and divided into two groups (n=6), Group I is Cosync protocol and Group II, is addition of insulin and metformin in Cosync and is termed as Co–ins–Sync protocol. All the buffaloes were examined by ultrasonography for the ovulatory status and fixed time breeding was performed. Plasma insulin and Thyroid Stimulating Hormone (TSH) was studied during day 0 and 9 of the treatment. In Cosync group one out of six buffaloes (16.6%) became pregnant, while two out of six (33.3%) buffaloes were pregnant in Co–ins–sync group. Significant increase in insulin and decrease in TSH values was observed on comparison with day 0 and 9 of the treatment in Co–ins–Sync group and attained the physiological reference range. From the present study, it could be concluded that insulin along with Cosync protocol ie., CO–INS'–SYNC would be a successful approach in postpartum anestrum buffaloes failed to conceive even after progesterone protocol.

Keywords: Anestrum, Buffalo, Cosync, 'CO–INS'–Sync, Insulin

Buffaloes are an important dairy animal and have a tendency in seasonal breeding; this results in quiescence of breeding activity during the summer season almost throughout the world. During this period, the animal may not exhibit evident estrous signs and is termed as summer anestrus (Das and Khan 2010). Reproductive hormones such as estrogen and progesterone in late gestation has negative feedback on hypothalamo-hypophyseal axis. Hence resumption of postpartum cyclicity is the effective response of hypothalamic-hypophyseal-ovarian-uterine axis. To establish an inter-calving period of 13–14 months, the estrus should be initiated by 60–80 days postpartum (El-Wishy 2007); suckling, nutrition, body condition score, milk yield, parity, season and other parturient abnormalities could influence the postpartum cyclicity in buffaloes. The incidence of postpartum anestrum (60–90 days) has been reported as 25.3% and 33.4%, respectively in Murrah buffaloes (Kumar *et al.* 2019, Mishra *et al.* 2023).

Various synchronization protocols have been used to improve conception in buffaloes and better results were found to be with progesterone based controlled internal drug release (CIDR) treatment (Raja *et al.* 2016, Kaurav *et al.* 2020). However, to address the synchronization failure in CIDR treatment, it is necessary to understand the importance of other metabolic hormones which are influencing the fertility. Thyroid stimulating Hormone (TSH) plays vital role in reproduction of animals and humans. It acts indirectly by several interaction with other hormones such as insulin growth factor (IGF), prolactin, gonadotrophin-releasing hormone (GnRH). Hence influence of TSH will cause subfertility (Silva *et al.* 2018). Metformin acts as an insulin sensitizer and also influences oocyte maturation and regulation of steroidogenesis (Bertoldo *et al.* 2014). The different genetic pattern of buffaloes on hormonal therapy is also playing a crucial role in choosing a right protocol for the treatment. Considering all these factors causing postpartum anestrum in buffaloes, insulin and metformin have been introduced in co–synch protocol to assess their efficacy in restoring the fertility of postpartum anovulatory anestrum in case of CIDR failure animals.

MATERIALS AND METHODS

Apparently healthy graded parous Murrah buffaloes (n=12) with 5–10 years of age with mean body weight of 422 kg which failed conceive in CIDR treatment

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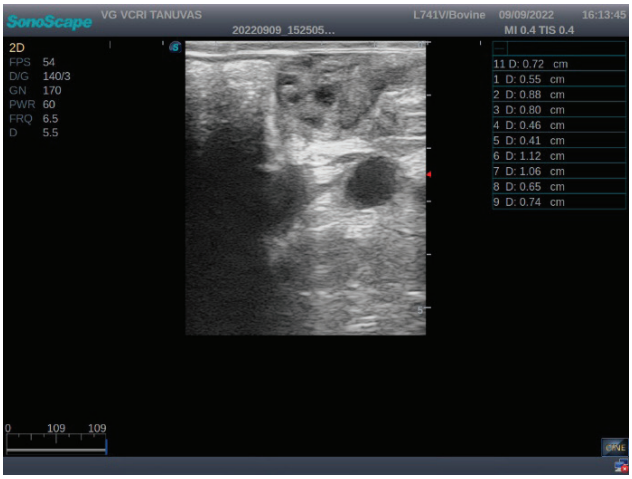

Fig. 1. Left ovary: Multiple small follicles

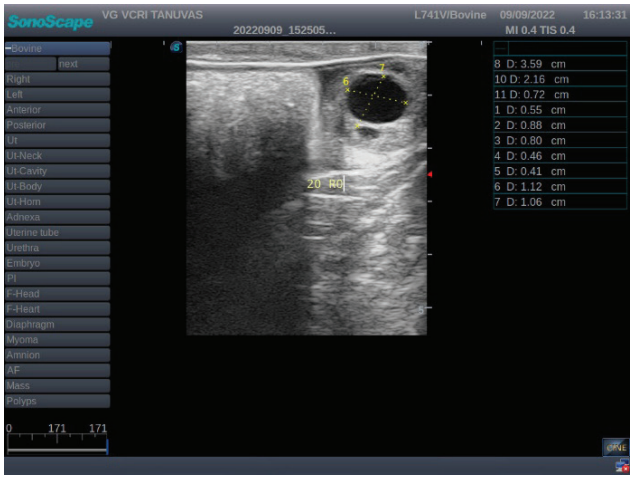

Fig. 2. Right ovary: Dominant follicle: 10.9 mm

were selected for the study. All the buffaloes were in the period of anestrum (75–120 days) after the CIDR treatment. The buffaloes were found to be normal, free from uterine infection and gross genital abnormalities with good body condition score. Rectal examination of the buffaloes revealed uterine tonicity (mild to moderate). The ultrasonographic examination revealed follicles (dominant along with multiple small follicles) without corpus luteum (Fig. 1 and 2). All the animals were maintained in the organized farm and ensured for concentrate feeding, green fodder, straw along with mineral mixture as per standard feeding. The selected buffaloes were divided into two groups; Group I (Cosynch protocol) were administered with Inj. GnRH analogue (Buserelin acetate 10 µg) on day 0 followed by Inj. Prostaglandin $F_2\alpha$ (Cloprosenol sodium 500 µg) on day 7 and 2nd dose of GnRH (10 µg) on day 9 intramuscularly. In Group II (CO–Ins–sync: Controlled Ovarian Insulin–Incorporated Synchronization), in addition to Cosync protocol, on day 0, the animal was administered with inj. Insulin@ 0.25 IU/kg subcutaneously and on day 1–5 Tab. Metformin HCl 1.5 g (3–5 mg/kg) per os was administered for 5 days consecutively. Five minutes prior to administration of Tab. Metformin, one per cent $CuSO_4$ solution was drenched to close esophageal grove for effective action of drug in true stomach. On day 9, fixed time breeding was practiced by Artificial insemination at 24 hrs interval in both groups. Blood samples were collected from all the buffaloes on day 0 and day 9. Plasma was isolated by centrifugation of blood sample at 2500 rpm for 10 min and stored at –20°C. Plasma concentrations of Insulin, TSH were determined using Enzyme–Linked Immunosorbent Assay (ELISA) method. All the buffaloes were examined for using ultrasonography on day 26 post insemination and conception rate was calculated

RESULTS AND DISCUSSION

In Cosync group, one out of six buffaloes (16.6%) became pregnant while two out of six (33.3%) buffaloes were pregnant in Co-ins-sync group. The plasma insulin and TSH values of individual animal and mean ±SE for the pregnant and non-pregnant animals in both groups are briefed in Table 1 and 2. Significant increase in insulin and decrease in TSH was observed with day 0 and 9 of the treatment in Co-ins-Sync group.

During the transition period, the biochemical profile gets altered and it has huge impact on postpartum cyclicity. Metabolic profile of postpartum animal could suggest the etiology for differential fertility (Jorritsma *et al.* 2003). Role of thyroid stimulating hormones in fertility is a complex mechanism which is regulated by altering steroidogenesis (Suriyasathaporn 2000). Altered thyroid activity has already been recognized as a prime cause for reproductive failure in female farm animals (Aggarwal and Singh 2010). Hypothalamus release thyrotropin-releasing

Table 1. Animal wise plasma insulin, TSH concentration and fertility status of buffaloes in Co–sync and CO–INS–SYNC group

CO–SYNC group					CO–INS–SYNC group				
Insulin ng/mL		TSH (mU/mL)		Fertility	Insulin ng/mL		TSH (mU/mL)		Fertility
Day 0	Day 9	Day 0	Day 9		Day 0	Day 9	Day 0	Day 9	
19.5	20.9	0.097	0.124	Non–Pregnant	19.6	34.5	0.113	0.042	Pregnant
12.2	13.5	0.081	0.074	Non–Pregnant	21.2	46.2	0.121	0.039	Pregnant
21.9	22.1	0.114	0.146	Non–Pregnant	17.6	18.3	0.064	0.069	Non–Pregnant
11.4	17.4	0.162	0.191	Non–Pregnant	22.3	19.2	0.069	0.062	Non–Pregnant
21.1	24.5	0.028	0.041	Pregnant	18.6	12.3	0.037	0.042	Non–Pregnant
18.6	26.3	0.247	0.192	Non–Pregnant	21.1	22.6	0.072	0.068	Non–Pregnant

Table 2. Mean ±SE plasma insulin, TSH concentration and fertility status in buffaloes of Co–sync and CO–INS–SYNC group

Pregnancy	CO–SYNC group				CO–INS–SYNC group			
	Insulin ng/mL		TSH (mU/mL)		Insulin ng/mL		TSH (mU/mL)	
	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
Pregnant	21.1	24.5	0.028	0.041	20.4±0.80	40.35±5.85	0.117±0.00	0.040±0.00
Non-Pregnant	16.72±2.08	20.04±2.17	0.14±0.03	0.14±0.02	19.9±1.09	18.1±2.14	0.06±0.01	0.06±0.01

hormone (TRH) in turn stimulate the pituitary to release TSH and it acts on thyroid gland to produce thyroid hormones (T4, L-thyroxine. T3, L-triiodothyronine) and maintaining the level of thyroid hormones in circulation. In contrast, hypothyroidism and hyperthyroidism has varying degree of decreased and increased activity at ovarian and uterus, respectively (Silva *et al.* 2018). TSH have unswerving potential effect on ovary activity and action in the granulosa and thecal cells (Mutinati *et al.* 2010). In addition to that, thyroid hormones could influence the pre and post implantation embryonic development. Furthermore, hypothyroidism could result in decreased fertilization rate in cattle (Ashkar *et al.* 2010).

Various studies had been reported direct or indirect action of metformin on ovary; although the mechanism of action is still not clearly understood. However, metformin action is having role in steroidogenesis activity at granulosa cell level, oocyte maturation, and also in gene expression of steroidal hormones (Bertoldo *et al.* 2014). Hence, administration of metformin ensures the release of competent oocyte which could enhance the fertility. Additional prime function of metformin is to decrease serum TSH from highly elevated range to moderately elevated level and reduce the size of small solid thyroid nodules (Karimifar *et al.* 2014). Similar findings were observed in the present study, where animals that conceived had higher TSH levels before treatment. Following treatment with the co-ins-sync protocol (group II), their TSH levels reduced to the normal range of 0.014 to 0.03 mU/mL, as reported by Kumar *et al.* (2010). Thyroid hormones can also influence the secretion and action of reproductive hormones and also get regulated by these hormones. This crosstalk would be one of the fundamental features in regulating fertility at wider range (Ren and Zhu 2022).

Changes in serum Insulin during the postpartum period will also reflect the same in follicular fluid which cause impairment in the oocyte quality, final maturation and ovulation. Increase or decrease in insulin level have an impairment in embryonic development and fertility. The difference in level of insulin is depend on the energy status of the animal and how it gets adapted to the response on metabolic homeostasis (Laskowski *et al*. 2014). Insulin administration might promote folliculogenesis as insulin like growth factors (IGF–I) and stimulate mitogenesis of the granulosa cell, thus resulting in increased post ovulatory progesterone production (Tiwari *et al.* 2014). In the present work, the level of insulin in pregnant animals had increased (Tiwari *et al.* 2014) to the normal range of 20.4±0.80 to 40.35±5.85ng/mL after insulin administration in group II.

However, effectiveness of hormonal therapy has its limitations in successful breeding practices of buffaloes. Higher circulating concentrations of growth hormone, β–hydroxybutyrate, endotoxin mediated diseases of uterus that have been associated with lower concentrations of insulin and TSH concentrations, which may be suggestive of low reproductive performance in postpartum buffaloes. The insulin and TSH levels need to be maintained in normal range to achieve fertility.

To conclude, most of the synchronization protocols till date are addressing effective estrus and ovulatory response, however the quality of oocyte is still questionable. Alteration in the level of TSH and insulin would cause subfertility. TSH cross talk with reproductive hormones and it gets further influenced by insulin. Metformin addresses both the insulin and TSH variations which, in turn, helps in release of the competent oocyte. To the best of authors knowledge, this report analyzed and highlights the importance on metabolic hormones such as insulin and TSH role in reproduction. Hence, administration of insulin and metformin in synchronization would help in improving the fertility in buffaloes. This approach facilitates validation and optimization, potentially enhancing the efficacy of the co–ins–sync protocol. Treating a larger cohort of animals, despite a success rate of less than 50%, is essential for refining the protocol, identifying factors influencing outcomes, and ensuring statistical robustness.

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