

Impact of insulin treatment during post-insemination mid-luteal phase on luteal profile and conception rate in buffaloes

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ABSTRACT

The aim of present trial in buffaloes was to test the impact of subcutaneous administration of insulin (long acting purified bovine insulin @ 0.2 IU/kg body weight/day) during post-insemination mid-luteal phase (days 8–12) on luteal profile and conception rate. A set of 22 estrus synchronized (2 PGF₂ α injections 11 days apart) post-partum buffaloes were assigned to insulin (n=12) or control (n=10) group. All the buffaloes were artificially inseminated (AI) 8–12 h after the onset of estrus and thereafter AI was repeated once at 12 h interval. Transrectal ultrasonography and jugular vein blood sampling was carried out on day 0 (day of last AI), 5, 8, 12, 15, 21 and 40 post-AI. First service conception rate was assessed by transrectal ultrasonography on day 40 post-AI. Data of insulin group showed no alteration in corpus luteum size ($P>0.05$), although post-treatment plasma progesterone and first service conception rate was higher ($P<0.25$) in insulin group compared to control group. In summary, insulin administration during post-AI mid-luteal phase may have a beneficial impact on conception rate in buffaloes.

Key words: Buffaloes, Corpus luteum, Conception, Insulin, Progesterone

About 20 to 40% of bovine embryos are lost within 22 days after breeding (Peters 2005). Luteal inadequacy during this period is one of the predominant factors responsible for embryonic mortality (Spencer *et al.* 2004). To compensate for such putative defect, insulin administration was used which stimulated growth and secretory activity of corpus luteum (CL) in cattle as well as optimized the embryonic development in goat (Selvaraju *et al.* 2002, Suguna *et al.* 2009). In buffaloes, evaluation of post-AI luteal profile and subsequent fertility following administration of insulin was not carried out. Thus, the objective of present trial in buffaloes was to test the effect of insulin administration during post-AI mid-luteal phase on luteal profile and first service conception rate.

MATERIALS AND METHODS

Animals: Postpartum buffaloes (n=22, body weight: 350–450 kg, age: 3–10 year, parity: 2–6, lactation yield: 2500–3000 litre) reared at private dairy farms were selected. These buffaloes have calved normally about 100–150 days ago and gynaecological examination at the start of trial revealed no gross abnormality of reproductive tract. Buffaloes were fed

green fodder, wheat straw and concentrates, and had access to *ad lib* drinking water.

Synchronization of estrus and AI: All the buffaloes were estrus synchronized using a double prostaglandin F₂ α (PGF₂ α) protocol. In this protocol, each buffalo was administered (i/m) 2 injections of 500 μ g cloprostenol at an interval of 11 days. Onset of estrus was detected through cervico-vaginal discharge (clear and thin), and was confirmed by the transrectal palpation of uterine tone. First AI with frozen thawed semen was done at about 8–12 h after the onset of estrus and thereafter second AI was carried out at 12 h interval.

Treatments: Control (n=10) received no treatment, whereas each buffalo of the insulin group (n=12) received a long acting bovine insulin (insulin zinc suspension IP, 40 IU/ml) @ 0.2 IU/kg body wt/day (Selvaraju *et al.* 2002). Insulin was administered subcutaneously once daily for five consecutive days during post-AI mid-luteal phase (days 8, 9, 10, 11 and 12).

Ultrasonography and blood sampling: Ovarian ultrasonography was carried out on day 0 (day of last AI), 5, 8, 12, 15, 21 and 40 post-AI. Ultrasonographic assessments for the diameter of largest follicle only on day 0 and for CL diameter on remaining days were made by the same person in all the buffaloes throughout the trial. Ultrasonography was carried out with a battery operated B-mode ultrasound

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scanner equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer (ALR 575 probe). Ovaries were examined systematically and images were drawn on a diagram of ovary by carefully sketching size and relative location of largest follicle (>10 mm), and size and location of visible CL. Optimal scan images were frozen and the size of the largest follicle/CL was determined by measurement of the diameter of the follicle/CL at their widest poles. All measurements were made using the built-in, on-screen calipers.

Blood was sampled from jugular vein in a heparinized vial after each ultrasonography. Plasma was separated immediately after blood collection and was frozen at -20°C until analysis.

First service conception rate: This was noted by diagnosing pregnancy status with transrectal ultrasonography on day 40 post-AI on non-return basis.

Hormone analysis: Plasma progesterone was assayed with the solid-phase radioimmunoassay using a progesterone antibody raised in our laboratory (Ghuman *et al.* 2007). Sensitivity of the assay was 0.1 ng/ml; intra-assay and inter-assay coefficients of variation were 6.2% and 9.5%, respectively.

Statistical analysis: Numerical data are represented as mean $\pm$ SEM. Between the control vs treatment groups, data for CL size and plasma progesterone were analyzed by independent *t*-test ($P<0.05$). Within groups, alterations in CL size and plasma progesterone on different days were evaluated using Duncan's Multiple Range Test ($P<0.05$). Chi-square (χ^2) test ($P<0.25$) was employed to compare the conception rate of control with treatment groups (Snedecor and Cochran 1994). These statistical procedures were performed using SPSS 16.0 computer package statistical software.

RESULTS AND DISCUSSION

This was the first study to investigate luteal profile in buffaloes following insulin treatment during post-AI mid-luteal phase in an effort to decrease embryonic mortality.

The results of this study suggest that, compared to control group, insulin treatment at the post-AI mid-luteal phase increased the first service conception rate in buffaloes ($P<0.25$, Table 1). In treated group, the conception rate was of higher magnitude (Table 1) compared to routinely observed in dairy herds (Spencer *et al.* 2004). Had the animal number been larger, the effect of insulin treatment on conception rate might have easily reached higher significance. Nevertheless, this distinct approach of injecting insulin at the post-AI mid-luteal phase increased conception rates which could be due to better functional activity of CL in terms of progesterone release. It was previously suggested that low progesterone during early embryonic development may cause pregnancy failure and thereby reduce conception rate (Lucy *et al.* 1993). Increasing peripheral progesterone between days 5 and 15

Table 1. Post-AI luteal profile and conception rate in buffaloes administered insulin during post-insemination mid-luteal phase (days 8–12). Day 0 is day of last AI

	Control	Insulin-treated
Largest follicle diameter, mm (day 0)	(n=10) 11.0 $\pm$ 0.3	(n=12) 11.0 $\pm$ 0.8
<i>Corpus luteum diameter, mm</i>		
Day 5 post-AI	12.62 $\pm$ 0.52	12.78 $\pm$ 0.91
Day 8	13.31 $\pm$ 0.54	13.78 $\pm$ 0.93
Day 12	13.29 $\pm$ 0.60	14.53 $\pm$ 1.04
Day 15	13.59 $\pm$ 0.76	13.84 $\pm$ 0.98
Day 21	12.82 $\pm$ 0.97	13.33 $\pm$ 0.78
Day 40	13.33 $\pm$ 0.45	13.82 $\pm$ 0.85
<i>Plasma progesterone, ng/ml</i>		
Day 0	0.06 $\pm$ 0.02 ^a	0.07 $\pm$ 0.03 ^a
Day 5	0.29 $\pm$ 0.09 ^{ab}	0.32 $\pm$ 0.06 ^{ab}
Day 8	0.47 $\pm$ 0.15 ^{abc}	0.70 $\pm$ 0.13 ^b
Day 12	0.69 $\pm$ 0.19 ^{*bcd}	1.26 $\pm$ 0.15 ^{*c}
Day 15	0.91 $\pm$ 0.21 ^{*cd}	1.47 $\pm$ 0.11 ^{*c}
Day 21	0.88 $\pm$ 0.22 ^{*cd}	1.37 $\pm$ 0.22 ^{*c}
Day 40	1.25 $\pm$ 0.20 ^c	1.68 $\pm$ 0.28 ^c
Conception rate (%)	20% [#] (2/10)	58% [#] (7/12)

$P<0.05$: values bearing different superscript in a column of a parameter; * $P<0.05$, # $P<0.25$, within a row.

of the estrous cycle may enhance embryo development and may suppress luteolysis, ultimately resulting in higher conception rate (Mann *et al.* 1999). Recently, it was reported that the production of interferon-tau (IFN-tau) and trophoblast length at day 16 post-AI was positively correlated more precisely with plasma progesterone on day 4 (Larson *et al.* 2007). This would suggest that the increase in maternal progesterone around day 5 might be required to further increase the conception rate. This warrants further studies in which post-AI luteal treatment can be planned during early-luteal phase.

As far as post-AI luteal profile is concerned, the present results of insulin group, compared to control group, do suggest stimulation of luteal activity in terms of plasma progesterone ($P<0.05$) despite the absence of difference ($P>0.05$) in CL diameter (Table 1). Plasma progesterone in insulin-treated buffaloes was higher ($P<0.05$) on day 12, 15 and 21 post-AI (Table 1). This might be due to direct stimulatory effect of insulin on CL leading to higher progesterone synthesis/release (Suguna *et al.* 2009). *In-vitro* studies utilizing bovine luteal cell culture also recorded insulin-induced increase in progesterone secretion (Sauerwein *et al.* 1992).

Nevertheless, on the day of estrus/AI (day 0), the absence of differences in diameter of largest follicle suggests similar follicle characteristics in buffaloes of both the groups (Table 1). In addition, on the same day, recording of negligible plasma progesterone (Table 1) suggests that

inseminations were carried out at the right time. In control group, a noticeably higher ($P < 0.05$) plasma progesterone observed on day 40 post-AI was the result of contribution from two pregnant buffaloes (Table 1).

In conclusion, administration of insulin during post-AI mid-luteal phase did not result in improved CL diameter, but was beneficial for the stimulation of progesterone release and improving conception rate. Hence, the present data suggest that luteal deficiencies following AI may be a major source of embryonic mortality in buffaloes.

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