



## Fertility response with four estrus synchronization regimens in prepubertal buffalo heifers

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Prepubertal anestrus and subestrus are the main conditions in which sexually mature buffalo heifers despite having attained appropriate body weight and age fails to exhibit overt estrus (Ghuman *et al.* 2008). Prepubertal heifers can be subjected to estrus synchronization regimens as they display follicular turnover in a typical wave-like pattern with dominant follicles attaining 12.2±0.3 mm diameter (Ghuman *et al.* 2010, 2012). The objective of present study in prepubertal buffalo heifers was to use estrus induction/synchronization as a potential alternative for abbreviating their age at puberty.

Prepubertal buffalo heifers (43) were randomly submitted to 4 estrus synchronization regimens: regimen 1 (n=11), ovsynch (20 µg GnRH analogue i.m. on day 0 and day 9, 500 µg cloprostenol sodium i.m. on day 7); regimen 2 (n=14), ovsynch plus controlled internal drug release (CIDR, 1.38 g progesterone) regimen in which CIDR was placed intra-vaginally from day 0 till day 7; regimen 3 (n=12), equine chorionic gonadotropin (eCG) 500 IU, i/m was administered on day 7 of regimen 2; regimen 4 (n=6), modified ovsynch in which day 9 GnRH was replaced by human chorionic gonadotropin (hCG) (3000 IU, i/m). All heifers were artificially inseminated (AI) on day 10 and, if required, on day 9 or day 11. Heifers returning to estrus were inseminated at spontaneous estrus. Conception rate was based on pregnancy diagnosis on day 90 post-AI. Jugular vein blood sampling and transrectal ovarian ultrasonography was done on day 0, 7, 9 and 10 (Ghuman *et al.* 2010). Largest follicle growth rate (mm/day) was the difference between largest follicle diameters on day 10 and day 7, divided by 3 (Sà Filho *et al.* 2010). Characteristics of induced-estrus were recorded between days 8 and 11. Heifers were observed for

visual signs of estrus, twice per day for 30 min. Scoring scale for observed symptoms of estrus was as per Singh *et al.* (2009). Estrus score of each day of each heifer was summed up and mean estrus score was calculated for each day of a group. Plasma progesterone was assayed with radioimmunoassay (Ghuman *et al.* 2009). Plasma 17β-estradiol (on day 9 and day 10), thyroid hormones (total triiodothyronine-T3, total thyroxine-T4 and thyrotropin-TSH, on day 0 and day 10) and blood metabolites (glucose, total cholesterol, triglycerides, total proteins, creatinine and blood urea nitrogen, on day 0 and day 10) were determined using ELISA kits. Numerical data differences were considered significant at P<0.05 using Chi-square ( $\chi^2$ ) test or Two sample Student's t-test.

The largest follicle diameter observed on day 0 in heifers of different regimens was ~10 mm (Table 1). Luteal status on day 0 (Table 1) revealed that 25.6% heifers were in subestrus and remaining were experiencing prepubertal anestrus. In response to first-GnRH, all the heifers (P<0.05) ovulated as indicated by the luteal status on day 7 (Table 1). The presence of higher plasma progesterone on day 7 in the CIDR-based regimens compared to remaining regimens (P<0.05, Table 1) could be due to supplementary contributions from the CIDR despite the release from the corpus luteum (CL, Table 1). A good synchrony in the stage of estrous cycle on day 7 was evident from the similar diameter of largest follicles on day 7 in various regimens (P>0.05, Table 1). In all regimens, heifers responded (P<0.05) to a day 7 PGF<sub>2α</sub> as revealed by the decline in plasma progesterone and CL diameter during the subsequent period of 72 h (P<0.05, Table 1). Concomitantly, the diameter of largest/dominant follicles observed in different regimens on day 7 continuously increased during the subsequent 72 h (P<0.05, Table 1). During this period (day 7–10), the largest follicle growth rate was numerically higher (P>0.05) in the heifers of regimen 2, 3 and 4 in comparison to regimen 1. Moreover, the largest follicle diameter on day 10 was higher in heifers synchronized with CIDR (P<0.05, Table 1). Progesterone has a positive effect on the follicular growth rate and diameter of the dominant follicle in prepubertal heifers (Sà Filho *et al.* 2010).

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Table 1. Ovarian response in prepubertal buffalo heifers (n=43) assigned to four estrus synchronization regimens

| Parameters                     | Regimens | Day 0                  | Day 7                    | Day 9                  | Day 10                 |
|--------------------------------|----------|------------------------|--------------------------|------------------------|------------------------|
| Largest follicle diameter (mm) | 1 (n=11) | 9.6±0.9                | 10.9±1.3                 | 13.3±1.0               | 14.2±1.2               |
|                                | 2 (n=14) | 9.8±0.8                | 11.8±1.2 <sup>a</sup>    | 14.8±0.5               | 16.1±0.7 <sup>b</sup>  |
|                                | 3 (n=12) | 8.2±0.8                | 10.6±1.0 <sup>a</sup>    | 14.4±0.7               | 16.9±0.6 <sup>b</sup>  |
|                                | 4 (n=6)  | 12.0±1.3               | 12.0±1.8                 | 14.2±0.9               | 15.8±1.4               |
| Plasma estradiol (pg/ml)       | 1        | -                      | -                        | 782.7±215.1            | 818.3±146.3            |
|                                | 2        | -                      | -                        | 503.8±196.3            | 576.2±188.8            |
|                                | 3        | -                      | -                        | 544.2±147.7            | 609.7±218.2            |
|                                | 4        | -                      | -                        | 933.3±217.9            | 889.1±297.4            |
| Corpus luteum diameter (mm)    | 1        | 2.25±1.5               | 10.4±1.0 <sup>a</sup>    | 4.8±1.5                | 1.2±1.2 <sup>b</sup>   |
|                                | 2        | 3.67±1.7               | 8.1±1.4 <sup>a</sup>     | 6.0±1.1                | 1.7±0.9 <sup>b</sup>   |
|                                | 3        | 4.33±1.9               | 8.2±1.8 <sup>a</sup>     | 4.0±1.7                | 0.7±0.7 <sup>b</sup>   |
|                                | 4        | 1.00±1.0               | 5.3±1.9 <sup>a</sup>     | 2.2±1.0                | 0.0±0.0 <sup>b</sup>   |
| Plasma progesterone (ng/ml)    | 1        | 0.17±0.07 <sup>a</sup> | 0.89±0.25 <sup>b,A</sup> | 0.29±0.11              | 0.17±0.08 <sup>c</sup> |
|                                | 2        | 0.23±0.10 <sup>a</sup> | 2.44±0.25 <sup>b,B</sup> | 0.26±0.10 <sup>c</sup> | 0.10±0.03 <sup>c</sup> |
|                                | 3        | 0.41±0.15 <sup>a</sup> | 3.02±0.45 <sup>b,B</sup> | 0.53±0.21 <sup>c</sup> | 0.27±0.09 <sup>c</sup> |
|                                | 4        | 0.08±0.05 <sup>a</sup> | 1.38±0.22 <sup>b,A</sup> | 0.31±0.09 <sup>c</sup> | 0.21±0.10 <sup>c</sup> |

<sup>a</sup> vs <sup>b</sup>P<0.05, within row of a parameter; <sup>A</sup> vs <sup>B</sup>P<0.05: within column of a parameter.

Table 2. Estrus behaviour *vis-à-vis* AI at induced-estrus in prepubertal buffalo heifers (n=43) assigned to four estrus synchronization regimens

| Parameters                          | Regimens | Day 0                  | Day 7                     | Day 9                    | Day 10                 |
|-------------------------------------|----------|------------------------|---------------------------|--------------------------|------------------------|
| Estrus score                        | 1        | 9.1±0.1 <sup>a</sup>   | 71.4±13.5 <sup>b,A</sup>  | 50.9±18.9 <sup>b,A</sup> | 26.4±16.3 <sup>b</sup> |
|                                     | 2        | 0.0±0.0 <sup>a</sup>   | 116.8±18.5 <sup>b,B</sup> | 137.9±6.9 <sup>b,B</sup> | 11.4±8.6 <sup>c</sup>  |
|                                     | 3        | 16.7±11.2 <sup>a</sup> | 140.8±14.0 <sup>b,B</sup> | 63.3±14.2 <sup>c</sup>   | 13.3±9.9 <sup>d</sup>  |
|                                     | 4        | 16.7±16.7 <sup>a</sup> | 100.8±22.1 <sup>b</sup>   | 91.7±30.0 <sup>b</sup>   | 0.0±0.0 <sup>c</sup>   |
| Heifers inseminated on various days | 1        | -                      | 9.1% (n=1) <sup>A</sup>   | 100% (11)                | 27.3% (3)              |
|                                     | 2        | -                      | 42.8% (n=6) <sup>B</sup>  | 100% (14)                | 21.4% (3)              |
|                                     | 3        | -                      | 58.3% (n=7) <sup>B</sup>  | 100% (12)                | 25% (3)                |
|                                     | 4        | -                      | 33.3% (n=2)               | 100% (6)                 | 0% (0)                 |

<sup>a</sup> vs <sup>b</sup>P<0.05: within row of a parameter, <sup>A</sup> vs <sup>B</sup>P<0.05: within column of a parameter.

Administration of eCG at CIDR withdrawal was aimed to augment the growth rate of follicles, thus leading to an increase in the diameter of the largest follicle at AI (Sà Filho *et al.* 2010). On the contrary, the diameter of largest follicle on day 9–10 was similar between regimen 2 and 3 (P>0.05, Table 1). Another potential problem with the synchronization regimens is the decrease in circulating estradiol prior to AI due to premature ovulation of the dominant follicles (Mann and Lamming 2000). Thus, it was also hypothesized that administration of eCG, because of its long half-life (~26 h) and capacity of binding to LH and FSH receptors, may stimulate the granulosa cells of dominant follicle to increase plasma estradiol (Kuran *et al.* 1996). On the contrary, plasma estradiol around the time of synchronized estrus in regimen-3 was similar to other regimens (P>0.05, Table 1).

Heifers with a CIDR device exhibited better estrus behaviour on day 9 and 24 h later (P<0.05, Table 2). Therefore, higher proportion of heifers in regimens 2 and 3 required AI on day 9 compared to regimen 1 (P<0.05, Table

2). Moreover, the percentage of heifers ovulating within 24 h of second-GnRH or its substitution by hCG was numerically higher (P>0.05) in regimen 2 (28.57%) and regimen 3 (16.66%) compared to regimen 1 (9.09%) and regimen 4 (0%). Also, the percentage of heifers conceiving in regimens 2 and 3 were numerically higher (P>0.05) compared to regimen 1 (Table 3). All these observations indicated that sufficient priming of hypothalamic system with progesterone during the pre-conception period may be necessary for a better display of estrus behaviour by heifers at induced-estrus, for the optimal growth of ovulatory follicle/subsequent CL as well as for the priming of endometrium to enhance the conception rate (Stevenson *et al.* 2006). The replacement of second-GnRH of Ovsynch protocol with hCG was based upon the hypothesis that hCG has long half life which will promote ovulation as well as lead to differentiation of granulosa cells to highly steroidogenic CL during post-AI luteal phase (Schmitt *et al.* 1996). Unexpectedly, none of the heifers of GP-hCG regimen conceived (P<0.05, Table 3).

Table 3. Conception in prepubertal buffalo heifers (n=43) assigned to four estrus synchronization regimens.

| Regimens | First service<br>conception rate (%) | Fate of non-conceived heifers |                            | Second service<br>conception rate (%) | Overall heifers<br>conceived (%) |
|----------|--------------------------------------|-------------------------------|----------------------------|---------------------------------------|----------------------------------|
|          |                                      | Remained anestrus (%)         | Returned to estrus (%)     |                                       |                                  |
| 1        | 18.2 (2/11)                          | 33.3 (3/9) <sup>A</sup>       | 66.7 (6/9) <sup>A</sup>    | 33.3 (2/6)                            | 36.4 (4/11)                      |
| 2        | 35.7 (5/14)                          | 44.4 (4/9) <sup>A</sup>       | 55.5 (5/9) <sup>A</sup>    | 40 (2/5)                              | 50 (7/14)                        |
| 3        | 33.3 (4/12)                          | 75 (6/8) <sup>b, B</sup>      | 25.0 (2/8) <sup>a, B</sup> | 100 (2/2)                             | 50 (6/12)                        |
| 4        | 0.0 (0/6)                            | 33.3 (2/6) <sup>A</sup>       | 66.7 (4/6) <sup>A</sup>    | 0 (0/2)                               | 0.0 (0/6)                        |

<sup>a</sup> vs <sup>b</sup>P<0.05: within a row. Figures in parentheses indicate number.

Fertility response observed in various regimens of current study was not influenced by their BCS, thyroid hormone status and blood biochemical profile as these parameters were within the physiological range and were similar between groups at the start of synchronization regimens and at the time of AI.

### SUMMARY

The use of four estrus synchronization regimens in 43 prepubertal buffalo heifers revealed that progesterone-based hormone regimens (ovsynch+CIDR±eCG) are most favourable for the optimization of their fertility status. Treatment with eCG at CIDR withdrawal had no additional beneficial impact on conception rate. Under field conditions, it may difficult to predict exhibition of induced-estrus/ovulation in prepubertal heifers, therefore, heifers subjected to Ovsynch+CIDR regimen should be inseminated at 48 (day 9), 72 (day 10) and 96 (day 11) h after CIDR withdrawal.

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