Use of Commercially Available Bovine Semen Sexing Agent in Buffalo: Preliminary Report of the Effect on the Conception Rate

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ABSTRACT

The aim of this work was to test the efficacy in buffalo of a post-thaw semen treatment product, called HeifersPlus™ (HP), available on the market for the bovine. This product works by enhancing the fertility of the X-chromosome bearing sperm and slowing the motility of the Y-chromosome bearing one, and attempts to alter the bovine sex ratio in favour of female. In this preliminary report we have evaluated the effect on conception rate (CR) after timed artificial insemination (TAI) comparing the use of HP treated semen with non-treated one in buffaloes synchronized with Ovsynch; moreover, we have evaluated the effectiveness of delayed insemination, testing the product either at 16h (standard protocol) or 24h (delayed protocol, as suggest by the manufacturer) TAI after GnRH. A total of 281 buffaloes were inseminated, 137 with HP treated semen (treated group) and 144 with no treated semen (control group). Significant differences were found in the CR, being 27.01% in the treated group and 42.36% in the control one (P≤0.007). The time of insemination (16h vs 24h) influenced the CR, being reduced at 24h in respect to standard 16h TAI. This was particularly evident in the control group where the CR was 50.88 and 36.78%, respectively at 16 and 24 h (P=0.09). In the treated group there was not significant differences in the CR between the two different time of AI (22.81 and 30.00%, respectively at 16 and 24h), however in both time of AI the observed CR was lower compared with the value obtained in the control group with no treated semen.

Keywords: buffalo, AI, semen sexing, conception rate

INTRODUCTION

Sexing technology takes advantage of the physiological differences between X and Y spermatozoa, favouring the presence of the desired kind of spermatozoa in the moment of fertilization and deviating the physiological sex ratio (Seidel, 1999). Although the basic principles controlling the sex of mammalian offspring have been known for a relatively long time, recent application of flow cytometry sorting system led to differentiation and separation of living X and Y chromosome-bearing spermatozoa (Garner and Seidel, 2008). However, a great disadvantage of this approach is the high cost of the semen doses and the reduced pregnancy rates when compared with no-sexed semen.

Recently, a post-thaw semen treatment product, called HeifersPlus™, is available on the market for the bovine. It works by enhancing the fertility of the X-chromosome bearing sperm and slowing the motility of the Y-chromosome bearing one, and attempts to alter the bovine sex ratio in favour of female. This product requires a delay in timing of insemination, therefore using Ovsynch protocol cows have to be bred 24h after GnRH.

The objective of work was to determine the efficacy of HeifersPlus™ (HP) in buffalo. In this preliminary report we have evaluated the effect on conception rate (CR) after timed artificial insemination (TAI), comparing the use of HP treated semen with non-treated one in buffaloes synchronized with Ovsynch.

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synchronized with Ovsynch; moreover, we have evaluated the effectiveness of delayed insemination, testing the product with TAI either at 16h (standard protocol) or 24h (as the manufacturer’s suggested) after last GnRH injection.

MATERIALS AND METHODS

The trial was carried out on 281 Italian Mediterranean buffaloes cows of different ages and parity, starting from February until May (low breeding season for buffalo reared in Italy). Buffaloes were divided into HeifersPlus™ (HP) (n=137) and Control (n=144) groups and subjected to oestrus synchronization and timed artificial insemination (TAI) using Ovsynch protocol. The treatment schedule was: 150 μg GnRH on day 0 + 0.15 mg cloprostenol (PGF2α analogue) on day 7 + 150 μg GnRH on day 9. After the last GnRH injection, two different time of TAI were used: 16h as standard protocol or 24h (as the manufacturer’s suggested). HP and Control were equally distributed in each TAI subgroups (16h n=57 HP and 57 Control; 24h n=80 HP and 87 Control). Buffaloes were inseminated using frozen-thawed semen of proved bull. In the HP group, after thawing, semen was added with the HeifersPlus™ and incubated in water bath at 37°C for 20 minutes before insemination, according to the kit instructions. The CR was assessed by ultrasound 28 days after TAI and confirmed at 42 days. Data were analyzed by χ² test (SAS/STAT User’s Guide).

RESULTS AND DISCUSSIONS

This is the first attempt to use the HeiferPlus in buffalo species. In this preliminary report the effect of treatment on the conception rate (CR) is presented. Significant differences were found in the overall CR of buffaloes confirmed pregnant at 42 day after TAI, between the group inseminated with semen treated with HeifersPlus (HP) and the group Control (Table 1). The treated semen resulted to give a lower fertility compared to the control being the CR 27.01% in the HP group and 42.36% in the Control group (P≤0.007). The embryonic loss, assessed by ultrasound at 42 day, was very low and was equally distributed between animal inseminated with treated and non-treated semen (data not shown); therefore the lower CR found in HP group cannot be attributed to embryonic mortality.

The HeiferPlus is a post-thaw semen treatment product that attempted to alter the bovine sex ratio in favour of females. The product works by enhancing the fertility of the X-chromosome bearing sperm and slowing the motility of the Y-chromosome bearing sperm. When inseminated, the sperm sort in the reproductive tract of the dam and the result should be more ova fertilized by the X-chromosome bearing sperm. Although the ingredients are undisclosed, the manufacturer published internet resources supporting its efficacy either on CR or on sex-ratio. They reported that the CR remained either unchanged or slightly better and sex ratio ranged from 67% to 100% heifer calves produced when HeiferPlus was used (field trial data 2006/2007; Emlab Genetic).

There are no reports on the use of HeifersPlus in species other than the bovine. The only scientific trial found in literature, that study the effectiveness of this semen sexing agent, is by Curry et al. (2009). The Authors reported that of the cows inseminated with control semen 48.0% became pregnant, while 54.5% of HP cows became pregnant. In contrast with the manufacturer data, no significant difference in sex-ratio between control and HP group was found by these Authors. To be more precise, Curry et al. (2009) used Ovsynch protocol to synchronize the animals, but inseminated 12h after the last GnRH that is early respect the suggestion of the manufacturer and this earlier insemination could be the explanation of the lack in sex-ratio shift.

In this work we wanted to evaluate, in addition to the attempt to obtain more female calves, the effectiveness of delayed insemination in CR testing the product either at 16h or 24h TAI after last GnRH injection.

From the results of our work seems that the time of insemination (16h vs 24h) influenced the CR, being reduced in 24h TAI respect to the standard 16h TAI for the Ovsynch program (Table 1). This was particularly evident in the Control group where the CR was 50.88 and 36.78%, respectively at 16 and 24 h(P=0.09). In the HP group there were not significant differences in the
CR between the two different TAI scheduled (22.81 and 30.00%, respectively at 16 and 24h), however in both time of AI the observed CR was lower compared with the value obtained in the control group with no treated semen. The CR obtained in the Control utilizing the standard Ovsynch procedure (16h TAI) shows that the animal respond positively to the oestrus synchronization treatment being the CR similar to those reported by others Authors (Barile, 2012).

At present, the only effective method for achieving sex pre-selection before conception, requires separation of X-chromosome from Y-chromosome bearing sperm and the flow cytometric technology is capable of producing sexed sperm at 90% of purity. The success of the technology depends mainly on the fertilizing capacity of the sorted semen that to date is still variable (Rath and Johnson, 2008). Thus, also using sexed semen the CR is reduced due to the separation process and the lower number of spermatozoa present in each sexed straw. In the bovine, where use of sexed semen for AI is being widely commercialized, a 20% reduction in conception rates compared to unsorted semen has been reported (Norman et al., 2010). The same reduction in CR was found in this work using HP treated semen. Therefore a reliable method of swaying the sex ratio in favour of female economically most convenient could be desirable in dairy industry, in bovine as in buffalo. If the results indicate from the HeiferPlus producer company in terms of female born will be corroborate, the product could be an inexpensive approach to the sex pre-selection of offspring, with the advantage to be used with the semen of whatever bull.

ACKNOWLEDGEMENT
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REFERENCES

Table 1. Conception rate (CR) in buffaloes inseminated with HeifersPlus™ treated (HP) or control semen in two different TAI time (16h and 24h after GnRH in Ovsynch program)

<table>
<thead>
<tr>
<th>TAI</th>
<th>HP Insemin No</th>
<th>Pregnant No</th>
<th>CR %</th>
<th>Control Insemin No</th>
<th>Pregnant No</th>
<th>CR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>16h</td>
<td>57</td>
<td>13</td>
<td>22,81**</td>
<td>57</td>
<td>29</td>
<td>50,88**</td>
</tr>
<tr>
<td>24h</td>
<td>80</td>
<td>24</td>
<td>30,00</td>
<td>87</td>
<td>32</td>
<td>36,78</td>
</tr>
<tr>
<td>Total</td>
<td>137</td>
<td>37</td>
<td>27,01*</td>
<td>144</td>
<td>61</td>
<td>42,36*</td>
</tr>
</tbody>
</table>

**P=0.002 ;*P=0.007 (in the row)