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Research Article
GnRH at Time of Insemination of Sexed Semen Enhanced Conception Rate in Holstein Heifers

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Abstract

Background and Objective: Exogenous GnRH at the onset of estrus or at time of insemination of conventional semen improves fertility. The current study aimed at evaluating reproductive performance of nulliparous Holstein heifers after insemination with sexed semen either with or without GnRH at time of insemination compared to conventional semen. **Materials and Methods:** A total of 300 heifers were randomly grouped and inseminated at their first natural estrus as follow: (1) Conventional semen (n = 100): inseminated with conventional semen straws (CRV, Netherlands), (2) Sexed semen without GnRH (n = 100): inseminated with sexed semen straws (SiryX®, CRV, Netherlands), (3) Sexed semen with GnRH (n = 100): inseminated with sexed semen straws combined with administration of 10 μg of Buserelin (GnRH agonist, Receptal®) by i. m injection at time of insemination. **Results:** Conception rate obtained by conventional semen group was significantly higher ($p<0.05$) than sexed semen without GnRH group (63 vs. 49%), while it was comparable to the value obtained by sexed semen with GnRH group (54%). Number of services per conception, gestational length and incidence of dystocia showed no significant difference among heifers ($p>0.05$). **Conclusion:** Therefore, conception rate of conventional semen is superior to sexed semen, however, administration of GnRH at time of insemination of sexed semen can improve its fertility output to a similar level.

Key words: Sexed semen, heifers, GnRH, conception rate, first lactation, insemination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

After several attempts and trials over many years, X and Y-chromosome bearing sperms were successfully separated in 1989 using flow cytometry[1]. Later on, cryopreservation of sex-sorted bovine semen paved the way for its commercialization for artificial insemination[2]. Implementing sexed semen for artificial insemination has enabled breeders to maximize herd profitability by providing adequate supply of replacement heifers. This is particularly advantageous for commercial milk producers because using sexed semen allow them to expand their herd with genetically superior daughters[3,4] and also to avoid production of surplus unwanted male calves, which is of low economic value for dairy breeders. Furthermore, commercial beef farmers can benefit of sexed semen by producing superior male offspring for growth and feed conversion efficiency for higher meat production[5]. In the field of embryo transfer research, sexed semen has been successfully used for insemination of super stimulated heifers and cows allowing the production of multiple superior heifers[6]. For non-domestic animals, sexed semen can help to retrieve small populations of endangered wildlife species in captive breeding programs[7].

The insemination dose of sexed semen will usually contain 2×10^6 sperm (vs. 20×10^6 sperm/dose in conventional semen), which is believed to provide an ideal balance between the cost of the sexed semen straw and acceptable field conception rate[8]. The sorting process is believed to compromise the sperm viability, motility and fertilizing ability[9]. In comparison to conventional semen, lower conception rates are achieved by using sexed semen, presumably because of fewer sperms in the insemination dose and lower sperm quality caused by sorting[10]. For these reasons, sexed semen was primarily recommended for application in virgin heifers to maximize cost-benefit ratio. Various approaches were employed to compensate the low fertility associated with sexed semen; some were based on increasing sperm dosage per insemination[10,11]. Others used sexed semen in conjunction with fixed time artificial insemination "FTAI"[12,13]. However, results varied among studies because other factors such as; accuracy of estrus detection and timing of insemination were also documented to affect conception rate of sexed semen[14,15].

Exogenous GnRH at the onset of estrus[16] or at time of insemination of conventional semen[17] was shown to improve fertility. Therefore, it was hypothesized that GnRH at time of insemination would improve the conception rate of sexed semen. On that basis, objectives of this study were to assess reproductive performance of Holstein heifers after insemination with sexed semen with or without GnRH at time of insemination compared to conventional semen and follow up the fertility parameters and milk production of these heifers at their first lactation season.

MATERIALS AND METHODS

Animals and management: The study was conducted on 300 nulliparous Holstein dairy heifers raised at a large commercial dairy farm located at Cairo-Alexandria desert road, north Egypt. Animals were housed in semi-open yards, sheds were supplied with a cool spraying system to be used in hot months. Animals were fed on total mixed ration (Table 1) that consisted of concentrates, corn silage, alfalfa hay, wheat bran, vitamins, minerals and calcium bicarbonate. Green berseem, berseem hay and wheat or rice straw were also fed whenever available, water was made available all the day time. All animals were vaccinated against FMD, lumpy skin disease and Rift Valley Fever Virus Vaccine and were under close veterinarian supervision to monitor general and reproductive health condition. All procedures used in this study were approved by the committee of scientific research ethics of Faculty of Veterinary Medicine, Alexandria University, Egypt.

Study design: The virgin heifers were enrolled in the breeding program over the period from 2016-2019. Natural estrus was detected by close visual observation of heifers by trained personnel twice per day for 30 min each. Heifers observed with signs of estrus were rectally palpated to confirm standing heat (ovulatory follicle and erected uterine horns) and then were artificially inseminated on the basis of the AM/PM rule by the same skilled veterinarian. According to the type of semen, heifers were allocated randomly to groups as follow: (1) Conventional semen (n = 100): inseminated with conventional semen straws (CRV, Netherlands), (2) Sexed semen without GnRH (n = 100): inseminated with sexed semen straws (SiryX®, CRV, Netherlands), (3) Sexed semen with GnRH (n = 100): inseminated with sexed semen straws combined with administration of 10 µg of Buserelin (GnRH agonist, Receptal®, Intervet/Schering-Plough animal health, USA) by i.m injection at time of insemination. Heifers returning to estrus were inseminated with a second and third insemination (3 cycles/heifer). Age and weight of heifers (mean±SD) at time of insemination was 393±10.2 ds/ 367±12.1 kg, 394±11.4 ds/ 372±12.6 kg and 396±11.4 ds/ 360±10.9 kg for conventional semen, sexed semen without GnRH and sexed semen with GnRH group, respectively. Majority of inseminations were performed during spring and winter (January-April).

Table 1: Composition of rations on dry matter basis

Nutrient*	Content
Dry Matter Intake (DMI) (kg)	10.5
Total Digestible Nutrients (TDN) (%)	59.3
Net Energy (NE) for maintenance (m) (Mcal/day)	7.49
Net Energy (NE) for gain (g)(Mcal/day)	1.77
Metabolizable Energy (ME) (Mcal/day)	22.5
Rumen Degradable Protein (RDP) (%)	9.1
Rumen Undegradable Protein (RUP) (%)	3.8
Crude Protein (CP) (%)	12.9
Calcium (g/day)	47.0
Phosphorus(g/day)	25.0

*The ration are devoted for animals of body weight (350-370 kg) with average daily gain = 0.5-1 kg

Pregnancy diagnosis and recorded parameters

Heifers: Inseminated heifers were checked for pregnancy by using ultrasonography 30-35 days post-insemination. For each animal group the following parameters were recorded: conception rate (3 inseminations), number of services per conception, gestational length, percentage of female calves born, weight of calves at birth and incidence of abortion/dystocia. Abortion was defined as pregnancy loss before 250 days of gestation, dystocia was considered when births were delayed and required forcible traction for delivering calves. Also, cows were monitored for 14 days after parturition to record signs of retained placenta and metritis. Retained placenta was considered when retention of fetal membranes continued for more than 12 h[18]. Metritis was diagnosed when cows pass fetid brown uterine vaginal discharge with elevated body temperature.

First lactation cows: Successfully parturient cows were visually inspected for signs of first postpartum estrus, animals were allowed for a Voluntary Waiting Period (VWP) between 44-50 days after calving. Insemination was performed upon detection of the second spontaneous pp estrus, whereas, cows that failed to show a second pp estrus were treated for FTAI using Presynch-Ovsynch program[19]. Only cows detected in estrus before end of the program were allowed for AI, while other cows were scheduled for timed AI 18 h after completion of the program. Inseminated females were checked for pregnancy by using ultrasonography 30-35 days post- insemination. Cows were machine milked by Delaval Alpha milking machine (40 head/parlor) three times daily at 8 h intervals starting at 06:00 am and milk yield were recorded for individual cows via computerized milking units. The following first lactation fertility and productive parameters were recorded for each group: days from calving to-first estrus, days from calving to 1st insemination, conception rate (2 inseminations), number of services per conception, days open, daily and 305-day milk yield.

Statistical analysis: All continuous variables passed the Shapiro-Wilk test for normality and hence, they were analyzed with univariate analysis of variance (ANOVA) with Tukey's test as *post hoc*. Quantitative data are presented as mean±standard error of mean. Categorical (count) variables are reported as percentages and compared among groups with the chi-square test. All analyses were performed with the SAS package[20] for Windows (version 9.1, SAS Institute Inc., Cary, NC, USA). The level of significance was set at $p<0.05$.

RESULTS

Fertility parameters of heifers: Data on conception and abortion rate of heifers are presented in Table 2. Results showed that total conception rate after 3 inseminations were significantly higher ($p<0.05$) in conventional semen than sexed semen without GnRH group (63 vs. 49%). Whereas, conception rate achieved by sexed semen with GnRH (54%) was statistically equivalent to the conventional semen group.

As shown in Table 3, number of services per conception did not differ significantly among groups. Both heifers that received sexed or conventional semen showed no difference in length of gestation (276±4.39 to 275±3.96 days) or weight of calves at birth (30.7±2.38 to 32.0±3.73 kg). Table 4 showed that percentage of female calves was higher in sexed semen without GnRH group (100%) and sexed semen with GnRH group (96.23%) than recorded in conventional semen group (37.7%). Incidence of dystocia/ metritis/retained placenta did not differ significantly among groups ($p>0.05$).

First lactation reproductive performance: Two heifers aborted in the conventional semen group while 1 heifer aborted in each of the other groups, thus, calving rate was 61% for conventional semen, 48% for sexed semen without GnRH and 53% for sexed semen with GnRH group. First lactation conception rate was not significantly different among groups (Table 5). Calving to first estrus interval was non-significantly shorter in sexed semen with GnRH group recorded at 22.7±7.76 days. Also, no difference was observed among groups in either calving to first insemination interval (55.7±3.59 to 56.1±3.95 days) or days open (71.5±10.5 to 73.8±7.69 days). Number of services per conception was non-significantly lower in sexed semen with GnRH group recorded at 1.75±0.44 straws. Type of semen did not affect daily milk yield or total milk yield at 305 day of heifers at their first lactation season (Table 6).

Table 2: Conception rates of heifers

Parameters	Conventional semen	Sexed semen without GnRH	Sexed semen with GnRH
Conception rate			
Total	100	100	100
Pregnant	63	49	54
Percentage	63	49*	54
Abortion			
Total	63	49	54
Aborted	2.0	1.00	1.0
Percentage	3.17	2.04	1.85

* Differs significantly ($p<0.05$) compared to the conventional semen, otherwise there is no difference ($p>0.05$)

Table 3: Fertility parameters of heifers

Parameters	Conventional semen	Sexed semen without GnRH	Sexed semen with GnRH
*Straws per heifer	1.87±0.72[a]	1.73±0.66[a]	1.85±0.74[a]
**Services per conception	1.86±0.74[a]	1.67±0.63[a]	1.67±0.75[a]
Gestation length (days)	275.00±3.96[a]	276.00±4.48[a]	276.00±4.39[a]
Calf birth weight (kg)	32.00±3.73[a]	31.10±2.89[a]	30.70±2.38[a]

Values are means±standard deviations, *All heifers (n = 100 per group), ** Pregnant heifers (n = 63, 49 and 54 for groups 1, 2 and 3, respectively), means with a common superscript letter in a row do not differ significantly ($p>0.05$)

Table 4: Percentage of female calves born and reproductive affections at first calving

Parameters	Conventional semen	Sexed semen without GnRH	Sexed semen with GnRH
Female calves (%)			
Total	61.00	48.00	53.00
Females	23.00	48.00	51.00
Percentage	37.70	100.00*	96.23*
Retained placenta			
Total	61.00	48.00	53.00
Positive	3.00	2.00	4.00
Percentage	4.92	4.17	7.55
Dystocia			
Total	61.00	48.00	53.00
Positive	8.00	8.00	6.00
Percentage	13.11	16.67	11.32
Metritis			
Total	61.00	48.00	53.00
Positive	9.00	5.00	7.00
Percentage	14.75	10.42	13.21

*Differs significantly ($p<0.0001$) compared to the conventional semen, otherwise there is no difference ($p>0.05$)

Table 5: First lactation conception rate

Parameters	Conventional semen	Sexed semen without GnRH	Sexed semen with GnRH
Conception rate			
Total	61.00	48.00	53.00
Pregnant	50.00	41.00	44.00
Percentage	81.97	85.42	83.02

Groups do not differ significantly ($p>0.05$)

Table 6: First lactation reproductive performance and milk production

Parameters	Conventional semen	Sexed semen without GnRH	Sexed semen with GnRH
Days to first estrus	23.5±7.40[a]	24.7±7.38[a]	22.7±7.76[a]
Days to first insemination	56.0±3.61[a]	55.7±3.59[a]	56.1±3.95[a]
Days open	73.8±7.69[a]	73.3±8.92[a]	71.5±10.5[a]
Number of services per conception	1.84±0.37[a]	1.80±0.40[a]	1.75±0.44[a]
Daily milk yield (kg)	29.3±2.01[a]	29.6±1.68[a]	29.8±2.07[a]
305-day milk yield (kg)	9083±640[a]	9182±549[a]	9224±662[a]

Values are means±standard deviations, means with a common superscript letter in a row do not differ significantly ($p>0.05$)

DISCUSSION

The main objective of this work was to evaluate the use of sexed semen with or without GnRH compared to conventional semen in dairy heifers. On-field reproductive performance of heifers was assessed post-insemination and also, at their first lactation season. Conception rate of conventional semen was significantly superior to sexed semen (without GnRH) recorded at 63 and 49%, respectively. Similarly, this result was shown by previous studies which indicated that fertility output of sexed semen is about 70-80% of conventional semen in nulliparous heifers[10,21,22]. Conception rate of sexed semen varies across studies, values up to 51[23], 40.2[21] and 31-42%[22] were reported. Even lower level of conception rate between 31.6 and 39% were shown by other studies[12,24,25]. Differences in sample size, inherent fertility of heifers and on-farm management system may explain different fertility outcomes of sexed semen.

The choice for including a GnRH dose at time of insemination of sexed semen in this study was an attempt to raise fertilization probability and hence, increasing its fertility return. The average interval from the onset of estrus to ovulation is about 27 h for dairy cattle[26]. Exogenous GnRH induces LH secretion, when given at insemination time it presumably could shorten the window between expected ovulation and timing of insemination. Findings showed that when heifers received GnRH they achieved comparable conception rate (54%) relative to heifers in conventional semen group. Although, low insemination dose of sexed semen is one reason of its decreased fertility, using higher number of sperms in the inseminating unit did not critically altered its conception rate[10]. The authors speculated that sperm dosage is not the main restrictive factor to the fertility outcome of sexed semen; rather it would be a matter of inseminator proficiency to properly handle semen throughout the insemination process. Interestingly, Sa Filho *et al.*[14] found that GnRH at time of estrus detection did not improve conception rate of sexed semen, but inseminating heifers 16-24 h vs. 12-16 h from onset of estrus increased conception rates. These results suggested that on-farm management strategies greatly affect fertility of sexed semen.

In the current study, percentage of female calves born for sexed semen without GnRH was 100 and 96.23% for sexed semen with GnRH; these values are higher than the majority of reports in literature which range from 85-90%[22,25,27], while Chebel *et al.*[21] reported percentage of female calves at 95.7% for one sire used in their work. The percentage of female calves for conventional semen in this study recorded at 37.7% was lower than previous studies that reported between 47.7 and 52%[21,22,25,27]. Parameters such as; gestation length, weight of calves at birth and incidence rates of abortion, dystocia, metritis and retained placenta did not show difference among heifers inseminated either with sexed or conventional semen; these results are in accordance with other researchers[21,27]. However, dystocia was described in other studies to be lower in first-calving heifers inseminated with sexed semen[22,28]. Percentages of heifers started their first lactation in this study were 61% in conventional semen, 48% in sexed semen without GnRH and 53% in sexed semen with GnRH group. First lactation conception rate of cows was not statistically significant among groups. Also, parameters of reproductive performance such as; calving to first estrus and to-first insemination interval, days open and number of services per conception did not differ among groups. De Vries *et al.*[4] described that 60% of dairy cows and heifers at breeding age are needed to produce sufficient number of replacements in herds using conventional semen. The equivalent value for sexed semen is still unknown, but it is believed that this number could be greatly reduced by the use of sexed semen in virgin heifers and high fertility profile cows[29].

Few studies in literature described the relationship between milk yield and the use of sexed semen in dairy farms. One study suggested that gestation of a female calf results in increased milk production specifically if it occurs at the first parity[30]. Nevertheless, in the current study, daily and total milk yield (305 days) of parturient heifers showed no difference among groups, These findings are in agreement with the study of Chebel *et al.*[21].

CONCLUSION

Findings of the current study indicate that administration of GnRH at time of insemination of sexed semen improved conception rate of virgin dairy heifers. This strategy may enhance the economic return/cost of using sexed semen for dairy breeders, therefore, expanding its use in dairy breeding system.

SIGNIFICANCE STATEMENT

Reproductive management of the herd can critically affect the fertility potential of sexed semen, this study showed that administration of GnRH at insemination time of sexed semen improved conception rate in nulliparous Holstein heifers. A great demand on using sexed semen in dairy herds is increasing to fulfill the need for dairy products across the globe, this study provide a practical strategy to enhance

fertility output of sexed semen in heifers. The strategy could enhance the economic return cost of using sexed semen for dairy breeders.

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