Research Article

Fertility of Commercial Sexed Semen and the Economic Analyses of its Application in Holstein Heifers

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Abstract | The aim of the current study was to evaluate the fertility of commercially available sexed semen and to economically analyse three assumed strategies for its application in Holstein heifers. In the first part of the study, a total of 426 heifers were inseminated with sexed semen from 7 bulls and 325 heifers were inseminated with unsexed semen from 5 bulls. The pregnancy at 40 and 90 days post insemination, the embryonic loss, the calving, the abortion and the heifer calves rates were calculated. Heifers inseminated with sexed semen had significantly lower (P<0.001) pregnancy-40, pregnancy- 90 and calving rates (34, 32.2 and 29.3%; respectively) than those inseminated with unsexed semen (62.5, 57.8 and 51.1%; respectively). The embryonic loss and abortion rates were similar in heifers inseminated with sexed and unsexed semen. There was a clear effect for bull on pregnancy, embryonic loss and abortion rates in sexed and unsexed semen. In the second part of the study, it was assumed that heifers will be inseminated with sexed semen once, twice or repeatedly till achieving 88% calving rate. The economic impact of the three sexed semen application strategies was compared to the unsexed strategy. The sexed once, the sexed twice and the sexed wide strategies achieved 8.7, 8.1 and 13.8% increase in the calf crop return; respectively. In conclusion, even with lower fertility, application of sexed semen could add numerous benefits to the dairy herds.

Keywords | Sexed semen, Reproductive performance, Economic analyses

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INTRODUCTION

Sexed semen is a new reproductive technology aimed to alter the sex ratio of the offspring toward a desired gender. About 90% of the new born will be from the required sex (DeJarnette et al., 2009). Bovine sexed semen was introduced into commercial application for the first time in the United Kingdom in 2000. Now a days, large number of artificial insemination centres offered bovine sexed semen worldwide (Rath and Johnson, 2008).The application of sexed semen extended to different species (Rath and Johnson, 2008; Lu et al., 2010) and it began to be used with other advanced reproductive techniques such as IVF (Pontes et al., 2010) and super ovulation (Hayakawa et al., 2009).

In expanding closed dairy herds, application of sexed semen facilitates faster, more profitable expansion of the herds. While in non-expanding dairy herds, sexed semen may be applied for production of replacement heifers for sale or to increase the value of beef output from the dairy herds (Hohenboken, 1999). Additionally, application of sexed semen could be an effective tool to improve the annual genetic gain (Sørensen et al., 2011; Khalajzadeh et al., 2012) through production of replacement heifers only from the superior genetics cows or higher chance to select the best genetic potential as replacement heifers (Sørensen et al., 2011; Khalajzadeh et al., 2012).

Limited application of sexed semen in developing countries may be attributed to its high price and low fertility (Seidel, 2003). The price of one sexed insemination dose is four times as the price of the unsexed one. The recorded sexed semen conception rate differed from herd to herd (DeJarnette et al., 2009) and from breed to another (Borchersen and Peacock, 2009). However, the cumulative data stated that the fertility of sexed semen is lower than that of unsexed one (Borchersen and Peacock, 2009; DeJarnette et al., 2009; Dominguez et al., 2011). About two-thirds of sexed semen fertility reduction was refereed to lower sperm number in the insemination dose (2-4 million), and the other third was refereed to the adverse effects of the sorting procedures (Frijters et al., 2009). The lower fertility limited the application of sexed semen to heifers those have higher predicted fertility (DeJarnette et al., 2009). This application may be limited to the first insemination or may be repeated to more inseminations. However, some recent reports recorded similar pregnancy rate after using sexed and non-sexed semen in Holstein heifers (An et al., 2010), non-suckling Nelore cows (Dominguez et al., 2011) buffalo heifers (Campanile et al., 2011), buffalo-cows (Lu et al., 2010; Campanile et al., 2013).

Despite of higher price and lower fertility, application of sexed semen had resulted in greater net profit than the unsexed semen at the end of time horizon (Hossein-Zadeh et al., 2010). The sexed semen conception rate and the extent of sexed semen application within the herd greatly affect the economic impact of sexed semen application. Therefore, the present study was designed to investigate the fertility of commercially available sexed semen and to economically analyse three assumed strategies for sexed semen application in Holstein heifers under Egyptians conditions.

MATERIALS AND METHODS

Heifer's Management and Examination

This study was carried out in ALQasem farm, Ismailia Road, Egypt in the period from September 2010 to December 2013. A number of 751 Nulliparous Holstein heifers were included in this study. Heifers were housed in a free stall and had a free access to water and feed ad libitum a total mixed well balanced ration. At 12 months age, the wither height and body weight were recorded. Heifers have body weight over 350 kg and wither height over 125 cm were judged as a mature heifers. All heifers were examined gynaecologically to exclude any genital tract anomalies. Heifers passed the gynaecological and body measure examination were proved for breeding.

Semen

All unsexed and sexed semen used in the current study were imported from American Breeding Service (ABS Global, Inc., Wisconsin, USA). Unsexed semen from 5 bulls (H,J,K,L,M) and sexed semen from 7 bulls (A,B,C,D,E,F,G) were used in the current study. According to the ABS sexation technical guide, the pre-freeze sexed sperm cell concentration is 2.1 million cells, and on average there are roughly one million motile sperm cells at thawing. While pre-freeze sperm cell concentration in unsexed semen was 25 million.

Estrus Detection and Artificial Insemination

Heifers were observed by professional heat observer all over the day. Heifers that showed standing estrus were introduced for examination 10-14 h later according to the AM/PM rules. Upon examination, heifers that showed turgid uterus and/or estrous mucus were inseminated either with unsexed or sexed semen. The semen was deposited into the uterine body by three skilled professional inseminators.

Pregnancy Diagnosis

The non-returned heifers were examined for pregnancy diagnosis at 40 days post-insemination and confirmatory pregnancy diagnosis was carried out at 90 days post-insemination. The pregnancy-40 and pregnancy-90 rates were calculated as the number of heifers diagnosed as pregnant at 40 and 90 days post-insemination -respectively- divided by the number of inseminated heifers. The embryonic loss rate was calculated as the number of heifers diagnosed as non-pregnant at day 90 divided by the number of pregnant heifers at day 40. The calving rate was calculated as the number of heifers gives birth after a complete pregnancy period divided by the number of inseminated heifers. The abortion rate was calculated as the number of heifers diagnosed as non-pregnant after confirmed pregnant at day 90 divided by the number of heifers diagnosed pregnant at day 40.

Economic Evaluation

Using the actual fertility indices of the commercially available sexed and unsexed semen, three assumed strategies for sexed semen application in heifers were economically analysed in comparison to unsexed strategy. In the first strategy (sexed once), it was assumed that heifers will be inseminated once with sexed semen. In the second strategy (sexed twice), it was assumed that heifers will be inseminated twice with sexed semen. In the first and second strategies, non-pregnant heifers after insemination with sexed semen will be inseminated with unsexed semen till achieving 88 % calving. In the third strategy (repeated sexed), it was assumed that heifers will be repeatedly inseminated with sexed semen till achieving 88% calving. In the unsexed strategy, it was assumed that heifers will be inseminated with unsexed semen till achieving 88% calving.

The number of heifers in all strategies was fixed to 100. The actual calving rate and the actual heifer calves ratio after sexed and unsexed semen were used to calculate the number of insemination required to achieve 88% calving and the suspected number of heifer calves in each strategy. The period between inseminations was adjusted to 30 days and it was used to calculate the suspected age at first calving and the suspected extra cost for rearing the heifers for longer period before the first calving.

Since the cost of rearing heifers from birth till breeding age is constant, only the cost of rearing heifers from breeding age till the first calving was taken in consideration. The suspected costs of insemination and rearing heifers for prolonged period in all sexed semen application strategies were compared to those suspected in the unsexed strategy. The suspected return from calf crop (pregnant heifers or fattened males) was calculated for all strategies. The percent of gain or loss after different sexed semen application strategies in comparison to unsexed strategy was calculated according to the following equations.

Return from heifers= Suspected number of heifers * price of the heifer in Egyptian pounds (LE).
Return from males = Suspected number of males * price of the male (LE)
Insemination costs = suspected number of required inseminations * price of the straw.
Additional costs from rearing heifers = Price of daily feed intake * days between each in two successive insemination.
Total return from the strategy = (Return from heifers + Return from calves) – (insemination costs + additional costs from rearing heifers)

Statistical Analysis

All statistical procedures were performed using SAS statistical system Package V9.2 (SAS, 2002). The proportion of dichotomous variables (pregnancy-40, pregnancy-90, embryonic loss, abortion, heifers calves) was evaluated with Chi-square test. Significant results were followed by multiple Z-tests to compare corresponding proportions. P-value for all pairwise comparisons was adjusted using the Bonferroni correction.

RESULTS

Fertility of Sexed Semen

Heifers inseminated with sexed semen had significantly lower (P <0.001) pregnancy-40, pregnancy- 90 and calving rates (34, 32.2 and 29.3 %; respectively) than those inseminated with unsexed semen (62.5, 57.8 and 51.1%; respectively). Similar embryonic loss and abortion rates were recorded in heifers inseminated with sexed (5.5 and 8.3 %; respectively) and unsexed semen (7.4 and 10.8%, respectively). The proportion of heifers calves after using sexed semen (89.6) was significantly higher than that after using unsexed semen (50; P <0.001) (Table 1).

The pregnancy-40, the pregnancy-90 and the calving rates were variable among different bulls in the unsexed (55.3 to 69.3, 51.6 to 66.7 and 44.7 to 56.1; respectively) and sexed (27.4 to 43.4 and 24.2 to 40.4 and 22.6 to 37.5; respectively) semen. The differences in pregnancy-40 and pregnancy-90 rates reach a significant level among sexed and unsexed bulls; respectively (P<0.05). The deviation from the average pregnancy-40 ratio was ranged from -6.4 to 9.6 and -1.4 to 8.5 in sexed and unsexed semen; respectively. The embryonic loss and abortion rates were significantly different among unsexed bulls (3.8 to 13.3 and 1.9 to 15.2; respectively) and sexed bulls (0 to 11.8 and 0 to 14; respectively). The sexed semen from bull B gave a significantly lower heifers calves ratio than other bulls (Table 2, 3).

Table 1: Reproductive performance of heifers inseminated with sexed and unsexed semen

Semen	Insemination	Preg-40[1]	Preg-90[1]	Embryonic loss[2]	Calving[1]	Abortion[3]	Heifer calves
Sexed	426	34[b]	32.2[b]	5.5	29.3[b]	8.3	89.6[a]
Unsexed	325	62.5[a]	57.8[a]	7.4	51.1[a]	10.8	50[b]

Values with different superscript (a, b) at the same column are significantly different (P<0.001). All values were presented as proportions;
[1]Pregnancy-40, Pregnancy-90 and calving rates were calculated as the number of heifers diagnosed as pregnant at 40 and 90 days post-insemination and number of heifers give birth after a complete pregnancy period respectively divided by the number of inseminated heifers;
[2]The embryonic loss rate was calculated as the number of heifers diagnosed as non-pregnant at day 90 divided by the number of pregnant heifers at day 40;
[3]The abortion rate was calculated as the number of heifers diagnosed as non-pregnant after confirmed pregnant at day 90 divided by the number of heifers diagnosed pregnant at day 40.

Table 2: Effect of bull on reproductive performance of heifers inseminated with unsexed semen

Bull	Insemination	Preg-40[1]	Preg-90[1]	Embryonic loss[2]	Calving[1]	Abortion[3]	Heifer calves
H	114	69.3	66.7 [a]	3.8 [b]	56.1	15.2 [a]	50
J	93	58.1	51.6 [b]	11.1 [a]	50.5	1.9 [b]	48.9
K	38	55.3	52.6 [a b]	4.8 [a b]	44.7	14.3 [a]	47.1
L	48	62.3	54.2 [a b]	13.3 [a]	45.8	13.3 [a]	50
M	32	59.4	65.3 [a]	5.3 [a b]	50	10.5 [a b]	56.3

Values with different superscript (a, b) at the same column are significantly different (P<0.05). All values were presented as proportions;
[1]Pregnancy-40, Pregannсy-90 and calving rates were calculated as the number of heifers diagnosed as pregnant at 40 and 90 days post-insemination and number of heifers give birth after a complete pregnancy period respectively divided by the number of inseminated heifers;
[2]The embryonic loss rate was calculated as the number of heifers diagnosed as non-pregnant at day 90 divided by the number of pregnant heifers at day 40;
[3]The abortion rate was calculated as the number of heifers diagnosed as non-pregnant after confirmed pregnant at day 90 divided by the number of heifers diagnosed pregnant at day 40.

Economic analysis of sexed semen application

The suspected age at first calving in the unsexed strategy (22.6 ± 0.73 m) was younger than that in different sexed semen application strategies (22.9 to 23.5, 22.9 ± 0.83 to 23.5 ± 1.5 m). The extra coast for rearing in the sexed once, the sexed twice and the wide sexed strategies were 1.7, 2 and 2.7 times as that in the unsexed semen strategy. Additionally, the cost of insemination required for achieving 88% calving in the sexed once, the sexed twice and the wide sexed strategies were 2, 4.4 and 6.9 times as that in the unsexed strategy. The number of heifers calves increased from 44 in the unsexed semen strategy to 56,64 and 80 in sexed once, sexed twice and sexed wide strategies; respectively. The sexed once, the sexed twice and the sexed wide strategies achieved 8.7, 8.1and 13.8% increase in the calf crop return; respectively (Table 4).

DISCUSSION

Under field conditions, it is difficult to compare the fertility of sexed and unsexed semen collected from the same bulls. The current study compared the fertility of commercially available sexed and unsexed semen and economically analysed three assumed strategies for sexed semen application in heifers.

Significantly lower sexed semen pregnancy rate recorded in the current study is in agreement with previous studies (Bodmer et al., 2005; Dominguez et al., 2011) but in disagreement with An et al. (2010). The sexed semen pregnancy rate recorded in the current study is around 55 % of the unsexed semen one. This is similar to results recorded by Bodmer et al. (2005) using Brown and Red Holstein heifers and some trials of Weigel (2004) and Schenk et al. (2009). However it is comparatively lower than many

Table 3: Effect of bull on reproductive performance of heifers inseminated with sexed semen

Bull	Insemination	Preg-40[1]	Preg-90[1]	Embryonic loss[2]	Calving[1]	Abortion[3]	Heifer calves
A	48	37.5 [ab]	37.5	0[b]	35.4	5.6 [b]	100[a]
B	97	28.9 [b]	28.9	0[b]	27.8	2.6 [b]	59.3 [b]
C	99	43.4 [a]	40.4	6.8 [ab]	34.3	14 [a]	91.2 [a]
D	57	29.8 [b]	26.3	11.8 [a]	22.8	11.8 [ab]	100 [a]
E	62	27.4 [b]	24.2	11.8 [a]	22.6	5.9 [b]	100 [a]
F	32	40.6 [a]	37.5	7.7 [ab]	37.5	0 [b]	100[a]
G	31	29.1 [b]	29.1	0[b]	25.8	11.1 [ab]	100 [a]

Values with different superscript (a, b) at the same column are significantly different (P <0.05). All values were presented as proportions

[1]Pregnancy-40, Preganncy-90 and calving rates were calculated as the number of heifers diagnosed as pregnant at 40 and 90 days post-insemination and number of heifers give birth after a complete pregnancy period respectively divided by the number of inseminated heifers

[2]The embryonic loss rate was calculated as the number of heifers diagnosed as non-pregnant at day 90 divided by the number of pregnant heifers at day 40

[3]The abortion rate was calculated as the number of heifers diagnosed as non-pregnant after confirmed pregnant at day 90 divided by the number of heifers diagnosed pregnant at day 40.

Table 4: Economic analysis for different sexed semen application strategies

	Unsexed[1]	Sexed semen		
		Sexed once[2]	Sexed twice[3]	Sexed wide [4]
No. of sexed straw*	0	100	171	298
No. of unsexed straw*	173	122	74	0
Cost of insemination	8640	26108	37861	59734
Increase in insemination cost**		2	4.4	6.9
Age at first calving (month)	22.6 ± 0.73	22.9 ± 0.83	23.2 ± 1.1	23.5 ± 1.5
Extra cost for rearing ***	22935	38486	45715	62582
Increase in the rearing cost**		1.7	2	2.7
Number of heifer calves	44	56	64	80
Number of bull calves	44	32	24	8
Net return from calf crop	1734563	1886093	1875434	1974454
Gain (loss) of sexed semen application**		+ 8.7	+ 8.1	+ 13.8

N.B. Costs and returned were calculated by Egyptian pound

[1]Unsexed: Heifers were inseminated with unsexed semen till achieving 88% pregnancy

[2] Sexed once: Heifers inseminated once with sexed semen then non-pregnant heifers after insemination with sexed semen were inseminated with unsexed semen till achieving 88 % pregnancy

[3]Sexed twice: Heifers inseminated twice with sexed semen then non-pregnant heifers after insemination with sexed semen were inseminated with unsexed semen till achieving 88 % pregnancy

[4]Sexed wide: Heifers repeatedly inseminated with sexed semen till achieving 88% pregnancy

*Number of straws required to achieve 88% pregnancy; **Calculated as comparison to the unsexed strategy; ***Extra coast for rearing from breeding age till first calving due to lower fertility previous reports stated that the fertility of sexed semen reached about 75-85 % of the unsexed semen (Garner and Seidel, 2003; DeJarnette et al., 2009; Borchersen and Peacock, 2009) and some trials of Seidel et al. (1999). The lower sexed/unsexed pregnancy proportion recorded in the current study is mainly due to comparatively lower sexed semen pregnancy rate that was about 20-30% lower than previous reports (Seidel et al., 1999; Borchersen and Peacock, 2009; DeJarnette et al., 2009). Since heifers that used in the current study received the same management including nutrition, housing, estrus detection, timing of AI as well as animal health programs and inseminated with the same inseminator. The lower sexed semen pregnancy rate may be due to difference in initial bull fertility or due to improper handling of the sexed semen itself during transportation of the sexed semen between different dealers.

Staining with Hoechst and exposure to UV during flow sorting tended to increase the incidence of sperm chromosome aberrations (Libbus et al., 1987). This was thought to affect fertilizing ability and embryonic survival after sexed semen application. In our study, we recoded similar embryonic loss and abortion proportions after using sexed and unsexed semen which is in agreement with some previous studies (Seidel et al., 1999; Borchersen and Peacock, 2009). This may be due to the fact that sperms with compromised DNA are removed during sorting procedures (Blondin et al., 2009; Gosálvez et al.,2011) and/or that the sperm sorting by flow cytometry improves the DNA integrity of the sperm cell population (Boe-Hansen et al., 2005). Bodmer et al. (2005) reported numerically higher proportion of embryonic loss after using sexed semen. However they used considerably few numbers of heifers.

The proportion of heifer calves after application of sexed semen recoded in the current study is in agreement with Bodmer et al. (2005) but lower than other earlier reports (Garner and Seidel, 2003; DeJarnette et al., 2009; Schenk et al.,2009) which indicates that the accuracy of the sorting procedures applied by the semen origin company is acceptable.

The fertility variations among bulls in the unsexed and sexed groups is in agreement with previous reports (Borchersen and Peacock, 2009; Frijters et al. 2009; Sales et al., 2010). These variations were attributed to different ability to penetrate the genital tract mucus and different ability to fertilize the oocytes (Al Naib et al., 2011) and/or different expression of some functional sperm specific proteins (Kasimanickam et al., 2012). Higher deviation from the average pregnancy-40 ratio among sexed semen bulls than unsexed semen bulls is in agreement with DeJarnette et al. (2009). Sexed semen may tend to magnify sire fertility difference that exist in unsexed semen due to sire variation in threshold sperm number required for optimum fertility (DeJarnette et al., 2009), improper selection of bulls those cannot achieve acceptable fertility with unsorted low dose (Rath and Johnson, 2008) and/or different ability to survive the sorting procedures (Borchersen and Peacock, 2009; Frijters et al., 2009; Schenk et al., 2009). The Clear effect of bull on the embryonic loss and abortion rates either in sexed and unsexed semen is in agreement with previous results (López-Gatius et al., 2002; Pegorer et al., 2007). Some bulls may have some recessive defects that adversely affecting embryo or fatal survival (VanRaden and Miller, 2006). Although most of sexed semen from different bulls achieved considerable high heifers calves ratio, sexed semen from bull B achieved comparatively lower heifers calves ratio. This may indicate a possibility for improper sorting procedures even with imported sexed semen form known company.

The fertility of sexed semen in comparison to the unsexed one is one of the important factors that greatly affect the economic impact of sexed semen application. The lower fertility of the sexed semen recorded in the current study resulted in great increase in the number of inseminations required to achieve 88% calving and subsequently increase the costs of insemination and rearing heifers for prolonged time before the first calving in all assumed strategies for sexed semen application. However, even with this higher coasts all sexed semen application strategies investigated in the current study had resulted in higher calf crop gain in comparison to unsexed strategy. The highest gain was recorded in the wide application strategy. Of course if the fertility of sexed semen is improved the economic impact will be maximized. If we added this economic benefit into the higher ability to expand the closed herd and the higher suspected genetic improvement of the herd, we can conclude that even with lower fertility, application of sexed semen could add numerous benefits to the dairy herds.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

All authors have been involved in designing the study, analysing the data and drafting the manuscript.

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