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Short Communication

FIRST RESULTS FROM INSEMINATION WITH SEX-SORTED SEMEN IN DAIRY HEIFERS IN MACEDONIA

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ABSTRACT

Science has been searching for a long time for a reliable method for controlling the sex of mammalian offspring. Recently, the application of specific modern cellular methodologies has led to the development of a flow cytometric system capable of differentiating and separating living X- and Y-chromosome-bearing sperm cells in amounts suitable for AI and therefore, commercialization of this sexing technology. The aim of this work was to present the first results of heifers that introduce bovine AI with sex sorted semen, for the first time in Macedonia. Insemination with sex sorted cryopreserved semen ($2x10^6$ spermatozoa per dose) imported from the USA was done at two dairy farms in ZK Pelagonija. In total, 74 heifers (Holstein Friesian) were inseminated. Inseminations were carried out in a timely manner following a modified OvSynch protocol. During the insemination, the sperm was deposited into the uterine horn ipsi lateral to the ovary where a follicle larger than 1.6 cm was detected by means of transrectal ultrasound examination. Pregnancy was checked by ultrasound on day 30 after the insemination. Overall, the average pregnancy rate in both farms was 43,24% (40,54% and 45,95%, for farm 1 and farm 2, respectively). All pregnant heifers delivered their calves following a normal gestation length (274,3 days in average) and of the 32 born calves, 30 (93,75%) were female. In conclusion, since the first results from inseminations with sex-sorted semen in dairy heifers in Macedonia are very promising, the introduction of this technique may bring much benefit to the local dairy sector. Average pregnancy rate seems similar with results obtained following 'regular' inseminations, notwithstanding the relatively low number of spermatozoa per insemination dose. Due to the latter, we however recommend inseminations only to be carried out by experienced technicians followinga TAI protocol and ultrasound examinations of the ovaries prior to insemination.

Key words: sperm sexing, heifer, ultrasound, artificial insemination

INTRODUCTION

For a long time, people were searching for a methodology that would be able to predetermine the sex of the offspring (13,15,16). For dairy cattle, this means production of female calves. Until recently, it was not possible to do that with a high accuracy.

In 1989, a major breakthrough in sperm sexing was reported. The USDA Beltsville Research Center group reported production of live offspring from sex-sorted, rabbit sperm (1). This was the first report of the birth of offspring of which the gender had been predetermined at conception by the use of living sperm sorted into the respective X- and Y-chromosome-bearing sperm cells. Sperm cells were stained with Hoechst 33342, sorted according to their DNA content, and subsequently deposited into the oviducts of rabbits (1). Insemination with sex-sorted Y-chromosome-bearing sperm resulted in 81% males (17/21), whereas insemination with X-chromosome-bearing sperm resulted in 94% females (15/16).

It is now possible to predetermine the sex of the offspring before fertilization in a number of species. The reported accuracy regarding the birth of offspring of the predetermined sex varies from 85 to 95% (2, 3, 4, 5).

Mature male gametes are small, haploid cells that can be accurately analyzed for DNA content

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because they are stable in healthy sperm. High resolution measurement of the DNA content of sperm was first achieved by flow cytometric analysis of demembranated spermatids or sperm nuclei (6, 7). The precision of this DNA measurement is such that the difference in DNA content between mammalian X- and Y-chromosome bearing sperm is detectable in a variety of species (7, 8). The initially used preparation process, however, severely damaged the sperm cells due to the aggressive removal of the tail and the membranes surrounding the nuclei prior to the staining with the membrane impermeable dye, 40-6-diamidino-2-phenylindole (DAPI) (7). It was only when the membrane permeable bisbenzimidazole DNA-binding dye, Hoechst 33342, was employed that accurate measurement of the DNA content was successfully achieved in living sperm (9). Precise measurement of the difference in DNA content between X- and Y-chromosome-bearing sperm of mammals has provided an effective means of separating viable gametes carrying either the X- or Y-chromosome with an accuracy of 85–95%. Flow cytometric sex-sorting of sperm according to their DNA content is patented (10) and has been sub-licensed for non-human mammals to XY Inc., through Colorado State University.

In domestic cattle, the chromatin of each somatic cell contains 60 chromosomes. Male gametes contain half that number because the haploid X-chromosome-bearing sperm that produces females carry 29 autosomes plus the X-chromosome. The haploid Y-chromosome-bearing sperm have the same 29 autosomes plus the male determining Y-chromosome. According to Moruzzi (11), the difference in total length of the bovine chromosomes than those from bulls and cows is approximately 4.2%.

Commercialization of sexed semen in the United States started in 2003 with a license granted to Sexing Technologies (ST). In February 2003, the first ST sexing laboratory started operations in Navasota, Texas.

Since Macedonia is still a country largely dependent on the import of female replacements for the further expansion of the dairy industry, our aim is to introduce bovine AI using sex sorted semen, in order to increase the number of internally raised heifers. The specific aim of the present paper was to report the results of the first inseminations using sex sorted semen in dairy heifers in Macedonia.

MATERIAL AND METHODS

Inseminations were performed on two dairy farms in ZK Pelagonija, using sex-sorted semen imported from the USA. As claimed by the company, each dose of sex-sorted semen contained 2.0 x 10^6 frozen spermatozoa. For that purpose, a total of 107 Holstein Friesian heifers were initially selected. Farms were large scale, with 500 cows each, and average milk production of 6200 liters per year. Cows and heifers in both farms are managed by routine husbandry procedures. The heifers included in the insemination schedule with sex-sorted semen were 15 months of age with at least 380 kg weight. Before the start of the estrus synchronization, all heifers were evaluated for their body development according to their age, and for ovarian functionality. Only 87 of the initially selected heifers were submitted to an OvSynch protocol before insemination with sex-sorted semen. Shortly, the protocol consisted of a basic GnRH treatment, followed by ultrasound examination (12) and PGF treatment (the latter only for heifers in which a functional CL – a CL with a diameter > 25 mm - could ultrasonographically be detected) and adding a second GnRH injection 60 hours after the PGF injection. This second GnRH injection induces ovulation of the dominant follicle recruited after PG-induced luteolysis. Before the second GnRH injection, another ultrasound examination was performed, and animals with follicles ≥ 16 mm were inseminated immediately, while the rest of them were checked again with ultrasound and inseminated at 12 hours after the second GnRH injection. Inseminations on both farms were carried out by the same experienced inseminator.

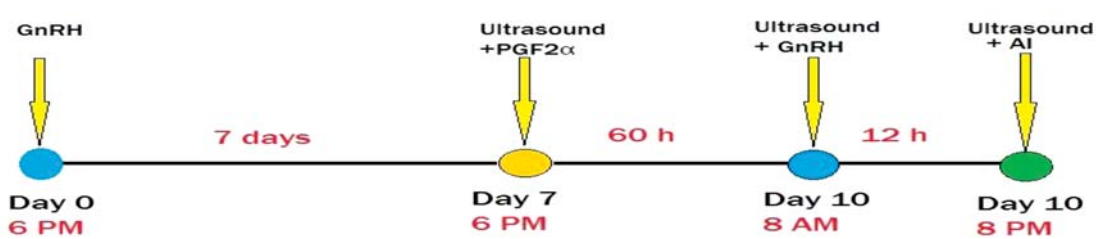

Figure 1. Modified OvSynch protocol for TAI of the heifers intended to be inseminated with sex-sorted semen

Out of 87 synchronized heifers, a total of 74 were inseminated. Heifers bearing small follicles <5 mm (n=13) on day 10 of the protocol, were considered as non-reactive to the treatment and were not inseminated. Insemination was done into the uterine horn ipsilateral to the ovary where a follicle larger than 16mm was ultrasonographically detected. Pregnancy was checked by ultrasound on day 30 after the insemination.

RESULTS

Data showing pregnancy rates for the heifers at both farms are presented in Table 1.

Reasonable pregnancy rates have been achieved with low-dose sex-sorted, cryopreserved sperm. The results varied between 25,00% to 71,43%, with individual variations within the farms from 0,00% to 71,43%. The only group with no pregnancy was on farm 1 during the winter season. We have to underline that within that group half of the prepared heifers were not inseminated due to problems with their condition or based on an unsatisfactory response to the treatment. The average conception rate for all groups was 43,24%, which is lower than the conception rate following insemination with conventional semen containing $20x10^6$ spermatozoa. At the same farms, heifers inseminated with conventional semen achieved an average pregnancy rate of 63,33%.

Table 1. Total results from insemination of heifers with sex sorted semen at 2 farms in Macedonia during the period of June 2012 – March 2013

Month	Total prepared heifers	Total treated heifers	Total inseminated heifers	Total pregnant heifers	% pregnant from inseminated
June	10	10	7	5	71,43 %
October	7	7	7	3	42,85%
November	14	9	8	2	25,00%
December	12	12	12	5	41,66 %,
January	10	6	6	3	50,00%
February	31	20	15	3	42,86%
March	23	23	19	11	50,00%
Total	**107**	**87**	**74**	**32**	**43,24%**

Table 2. Percentage of the female calves from the calved heifers inseminated with sex-sorted semen during June 2012 – March 2013

Month	Total inseminated heifers	Total pregnant heifers	% pregnant from inseminated	Born female calves	% females
June	7	5	71,43 %	5	100%
October	7	3	42,85%	3	100%
November	8	2	25,00%	2	100%
December	12	5	41,66 %,	5	100%
January	6	3	50,00%	3	100%
February	15	3	42,86%	2	66,66%
March	19	11	50,00%	10	90,90%
Total	74	32	43,24%	30	93,75%

All the pregnant heifers delivered their calves following a normal gestation length and 30 of the 32 (93,75%) born calves were female (Table 2).

From a total of 32 pregnant heifers inseminated with sex-sorted semen, 30 gave birth to a female calf, while only 2 of the calves were males. The latter implies an accuracy of the gender prediction of 93,75% (ranged from 66,66 to 100,00%).

DISCUSSION

The results mentioned in the present report, are as predicted by the semen company (pregnancy rate >40% and >90% female calves), and are similar with results mentioned by other authors (13, 14, 17).

Seidel and Garner (13) reported on their large scale experiment (371 heifers were inseminated during 3 days) an average pregnancy rate of 53%, using two different doses of sexed spermatozoa (1,5 x 10^6 and 4,5 x 10^6 frozen spermatozoa per insemination dose), with no significant difference.

Results from several trials conducted by XY, Inc. in Colorado (2) show that pregnancy rates with unsexed control semen were 74% at pregnancy examinations carried out at 30 to 33 d after insemination and 69% at reexaminations at 64 to 67 d after insemination. Pregnancy rates with sexed semen at 30 to 33 d after insemination ranged from 48 to 55%, but the effects of sperm concentration (1.5 vs. 3 million per straw) and semen placement (uterine horn vs. uterine body) were minimal. Pregnancy loss between 30 and 33 d and 64 and 67 d after insemination with sexed semen, ranged from 2 to 7%, and was similar to the rate observed using conventional semen. These results are similar with our results although we observed a variation in final pregnancy rates from 25,00% to 71,43 %. This rather big variation could mainly be attributed to problems with one group of the heifers. In that particular group, 18,75% of the originally selected animals were excluded even before the implementation of the OvSynch protocol, while a further 31,25% were not inseminated due to poor development of the follicles. When this group should be excluded, the pregnancy rate was on a satisfactory level. The same conclusion was obtained from Garner and Seidel (14). In their review on the history of the commercialization of sexed semen for use in cattle, they pointed out that among 25 herds, where more than 100 doses of sexed semen were used (608 - 122 inseminations/herd), conception rates averaged 48.2% and ranged from 33 to 72%. They argued that these rather large variations in results between different herds indicate that differences in management level at the herd are of decisive importance in realizing satisfactory results.

CONCLUSION

In the present paper we report the first results obtained from inseminations with sex-sorted semen in dairy heifers in Macedonia.These results are very promising and will have much benefit to the further development of the local dairy sector.

Average pregnancy rate was as could be expected taking into acountthe relatively low number of spermatozoa per insemination dose.

Due to this fact, we recommend inseminations only to be carried out by experienced technicians applying the TAI protocol and under strict ultrasound control of the ovaries prior to insemination.

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