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First field results on the use of stallion sex-sorted semen in a large-scale embryo transfer program

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ABSTRACT

Flow cytometry sex-sorting technology was developed in 1989. However, it is only the bovine species in which offspring of the desired sex are obtained at a commercial level. The aim of the present work was to evaluate efficiency parameters when using fresh sexed semen in a large-scale equine commercial embryo transfer program. During the 2009, 2010 and 2011 breeding seasons, 938 synchronized cycles were artificially inseminated. One hundred (10.6%) mares failed to ovulate, and for the remaining 838 useable cycles, 887 doses of sexed semen were used, representing 1.06 doses per cycle. In general, 435 (51.9%) out of 838 flushing performed resulted in the recovery of at least one embryo and 496 (59.1%) embryos were recovered, including twins and triplets. Pregnancy rate at 25 days achieved 81.5% (one embryo transferred per recipient). Embryo recovery rate was not statistically different either between preovulatory and postovulatory artificially inseminated mares or when increased quantities of sexed sperm per dose were used (15–45 million) ($P > 0.05$). A broad variation in embryo recovery rate was observed between the different stallions used in this study. Sex accuracy of the sex sorting assessed by ultrasound fetal sex determination was 90.3%. Finally, overall efficiency (female embryo pregnancies per useable cycles) was 39% (325/838), meaning that to obtain a female pregnancy of at least 75 days it was necessary to perform 2.5 flushing.

1. Introduction

Flow cytometry sex-sorting technology was developed in 1989 [1]. Since then, offspring of many different species such as sheep, bovine, rabbit, pig, horse, deer, dog, cat, and dolphin has been reported [2]. However, it is only the bovine species where offspring of the desired sex are obtained at a commercial level encouraged by a demand for females for dairy farming [3,4]. The number of sperms that can be effectively sex-sorted per hour per flow sorter was a limitation to the application and widespread use of the technology in species that require large number of sperms to inseminate the female such as the pig and horse species. In the pig, this limitation was partially overcome using a combination of a reduced number of sex-sorted sperm and a laparoscopic procedure to deposit the semen directly into the oviduct [5]. In the equine case, few works focused on the combination of flow cytometry sorting technology and hysteroscopic insemination procedure, in which a reduced quantity of sexed sperm (fresh or frozen-thawed) was deposited near the uterotubal junction [6–9]. A combination of large pipette and deep intrauterine artificial insemination (AI) with stallion-sexed semen was recently reported as a valid alternative technique to avoid the time consuming procedure and technical training necessary to perform the hysteroscopic procedure [10].

Preselecting the sex of the offspring is extremely important for some horse breeds, such as polo mares, in

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which most of the horses playing polo are females; therefore, sex selecting for female offspring is highly desired in this breed. In general, during every breeding season, these top player mares are submitted to an embryo transfer (ET) program as donors, and while they are producing embryos, gestations are carried by surrogate recipient mares so that player mares can go back to sport activities once the breeding season comes to an end [11]. As a country, Argentina was the second in the world in the number of flushing performed to mares, according to the International Embryo Transfer Society 2010 annual report [12]. Therefore, setting up a protocol to combine AI and ET techniques using sexed semen could be a very interesting option for polo mare producers because management schemes for embryo production benefit from sex preselection based on the ability to plan mating for a specific sex. However, the application of this technology to equine is dependent on economics, efficiency, and ease of use. Therefore, the aim of the present work was to evaluate efficiency and factors that may influence the embryo recovery when using sexed semen technology under field conditions in a large-scale commercial ET program.

2. Materials and methods

During the 2009, 2010 and 2011 breeding seasons, our laboratory produced sexed semen for Embryo Transfer Centers (ETCs). Two different operational systems were used; the ETC brought mares (donors and recipients) and stallions to our farm and laboratory, and we moved one or two sex-sorter machines to the ETC using a trailer adapted as a mobile laboratory. In both cases the routine was similar; semen was collected by the ETC personnel, then sexed and packaged in our laboratory and finally delivered to the ETC veterinarian who performed the insemination.

2.1. Mares management

Donor mares synchronization: once a mare has initiated estrous, it was daily ultrasonographically monitored to measure follicle size and its growth. Once the follicle achieved at least 35 mm, ovulation was synchronized using 1.500 UI of hCG (Ovusyn; Syntex, Argentina) injected intravenously (iv) in the jugular vein. Then, mares were similarly controlled to those protocols developed for frozen semen i.e., one ultrasound every 6 to 8 hours till imminent or recent ovulation was diagnosed [13]. In this study, AI was performed within 12 hours before until 6 hours after ovulation was ultrasonographically diagnosed. Mares were not inseminated at fixed time after hCG treatment. So, after receiving hCG, the order of priority of mares to be inseminated was updated every 6 to 8 hours according to individual preovulatory follicle development. Follicle size, shape, texture at palpation, and ultrasonographic patterns of the follicle fluid were used as indicators of proximity to ovulation. Additional inseminations were performed if the mare did not ovulate within 12 hours after first insemination was made.

2.1.1. Insemination

Sexed semen was nonsurgically deposited deep in the uterine horn ipsilateral to the preantral or the recent ovulated follicle using a long flexible pipette (Minitube, Verona, WI, USA) and following the rectally guided deep horn insemination technique. In brief, the pipette is moved by rectal palpation toward the tip of the uterine horn, once the pipette is placed in the desired position, an assistant helped in releasing the semen by pressing the steel plunger, which moved the cotton plug to the open end of the straw, thus pushing the semen close to the uterotubal junction [14].

2.1.2. Embryo collection and classification

Between 8 and 10 days after ovulation, all mares were flushed following the standard procedures [15]. Embryos were searched and manipulated following International Embryo Transfer Society procedures, and once classified according to the stage of development, they were charged into a 0.5-mL straw, one embryo per straw. Only embryos graded as quality 1 ($n = 481$; 97%) and 2 ($n = 15$; 3%) were used for the present study.

2.1.3. Recipient synchronization and ET

Mares accepted as recipients for the ET program were between 4 and 12 years old. They were daily monitored to determine estrous initiation using a teasing stallion, then all recipients diagnosed as in estrous were daily scanned to follow up dominant follicle growth till ovulation. Recipients that ovulated 1 day before (+1) or 0 to 3 days (0–3) after the donor mare were selected as suitable synchronization for ET. A single embryo was transferred into the right uterine horn regardless the location of the CL.

2.1.4. Pregnancy diagnosis

Six days after ET, recipients were scanned for pregnancy; presence of the embryonic vesicle was used as a positive indicator of pregnancy. A second ultrasound was subsequently done at 25 days to confirm viability of the embryo through positive heart beating sign.

2.1.5. Fetal sex determination

When possible, mares were made to fast at least 8 hours before examination. Between 59 and 78 days of gestation, a single transrectal ultrasound examination was performed by the same operator using a real-time diagnostic scanner equipped with a linear-array 5-MHz transducer (Aloka SSD 500; Aloka, Tokyo, Japan). A well-established protocol was followed [16]. In brief, with the standing mare, manure was manually removed, the probe was gently covered with gel and introduced deep in the rectum till the uterus was seen on the screen. Once the fetus was located, the operator scanned a cross section of it from head to tail. If the fetus was a male, a hyperechoic bilobed structure (male genital tubercle) was imagined immediately behind the umbilical cord abdominal insertion. When the male genital tubercle did not appear at this location, the operator continued scanning the fetus to visualize the female genital tubercle under and just cranial to the tail.

2.2. Semen collection and sex processing

2.2.1. Semen collection

Nine hundred and eighty-five ejaculates from 40 different Polo breed stallions were collected using a