Rocz. Nauk. Zoot., *45*, *1* (2018) 3–8

SEXED SEMEN. REASONS FOR REDUCED FERTILITY AND THE POSSIBILITY OF ACCURATE QUALITY ASSESSMENT*

Piotr Gogol

National Research Institute of Animal Production, Department of Reproductive Biotechnology and Cryoconservation, 32-083 Balice near Kraków, Poland

The use of sexed semen is highly attractive for cattle breeders who want to considerably increase the number of female calves born in a herd. Today, the proportion of female calves obtained after insemination with X-bearing sexed semen is around 90%. Semen-sexing technology is subject to continuous improvement, but it still offers lower conception rates (fertility) compared to conventional semen. The main reason is reduced quality of spermatozoa, which are exposed to technological stress factors during sexing. Therefore, it is of paramount importance that their in vitro quality can be accurately assessed. Standard sperm assessment methods such as motility, morphology and survival have many limitations, and their results correlate with sperm's fertilizing capacity only to a certain extent. For this reason, it is necessary to use modern semen assessment methods to provide a more accurate basis for determining semen fertility in vivo.

Key words: sexed semen, fertility, cattle

An interest in methods of animal sperm separation has been noted since the wide introduction of insemination to cattle breeding practice. At the beginning, the main barrier was lack of reliable and rapid methods of identification of sperm "gender" and the possibility of effective separation of the recognised fractions. In the majority of mammal species, the Y chromosome is the smallest or one of the smallest elements in the whole set. The majority of methods that have been applied to recognize the X and Y chromosome-bearing spermatozoa consisted in using the differences in structure and size between these two chromosomes.

For some time, the most popular has been the possibility of spermatozoa separation using flow cytometry. An idea of applying it to sperm separation according to "gender" arose in the early 1980s, when Pinkel et al. (1982) discovered a possibility of precise measurement of DNA and differences of its content between the X- or Y-chromosome bearing spermatozoa. The investigators stained the fixed spermatozoa from several mammal species with ethidium bromide, mithramycin or

* The study is financed by the statutory funds of the National Research Institute of Animal Production, task number 01-19-06-11.

DAPI (4',6-diamidine-2-phenylindole). Similar studies with the use of Hoechst 33342 fluorochrome (bis-benzimidazole) were conducted by Keeler et al. (1983). This dye, which is currently used in semen sorting, easily penetrates through the undamaged membranes of living cells, stoichiometrically binds to DNA in the region of A-T base pairs, and fluorizes at wavelength of 450 nm after induction with ultraviolet light.

Completely effective separation of spermatozoa was performed in rabbit semen in the late 1980s (Johnson et al., 1989). Re-analysis of fresh rabbit semen stained with Hoechst 33342 demonstrated that the accuracy of separation amounted to 86% in case of "female" spermatozoa and 81% in case of "male" spermatozoa. The separated spermatozoa were surgically introduced to uterus of does. Approximately 28% of births were obtained and the offspring born after the insemination with the X fraction was in 94% of female gender and after the insemination with the Y fraction – in 81% of male gender. Slightly worse results were achieved in case of sorting boar semen (Johnson, 1991). In this case, the effectiveness of separation was assessed based on re-analysis and surgical insemination of sows as well. The percentage of female piglets after insemination with the "female" fraction amounted to 74%, whereas the percentage of male piglets after the insemination with "male" fraction was – 68%. The above-mentioned studies demonstrated that there is a real possibility of sperm separation according to the determined sex. However, at the same time, these studies indicated that there are some significant restrictions of the method, among which the most important was quite low sorting speed, amounting to on average 100–200 cells/second. Apart from low effectiveness of sorters themselves, this speed was also indirectly affected by a specific, flat shape of spermatozoa head that caused a phenomenon of uneven optical density and some kind of fluorescence lensing. It consists in diffraction of fluorescence derived from the inside to the edges of spermatozoa head. Thus, the fluorescence is not evenly distributed around the whole head, but is lensed in directions close to is surface. In case of quantitative analyses of spermatozoa DNA, where the expected differences in fluorescence are slight, this phenomenon is extremely unfavourable. Spermatozoa flowing in the point of analysis stand towards the detectors randomly, so differences in fluorescence intensity resulting from its lensing completely "extinguish" the differences caused by the actual amount of DNA in a cell. This phenomenon required the application of special modifications to flow cytometers consisting in a change of shape of the exhaust nozzle and reading the fluorescence of spermatozoa DNA by two detectors set at the angle of 90°, simultaneously. The nozzle modified in such a way caused flattening of the stream of liquid with spermatozoa to the form of a ribbon, so their heads were set mainly on the same surface, whereas transferring the detector enabled to read DNA fluorescence also from the remaining part of "improperly" set spermatozoa.

The effect of a dye and laser light inducing it on fertilizing potential of sexed spermatozoa and further embryo development is still an important issue. As it has been already mentioned, the fluorochrome applied for semen separation is Hoechst 33342, induced by UV at wavelength of 350 nm. In bull spermatozoa stained with

the use of this compound, a slight decrease in the ability to *in vitro* insemination was observed, but it did not concern all the analysed ejaculates. However, the initially raised reservations concerning chromosome damages or offspring defects as a result of insemination with stained spermatozoa were not confirmed (Smorąg et al., 1993). Also, the studies on the effect of UV light beam on spermatozoa did not confirm the previous reservations (Guthrie et al., 2002).

Currently used flow cytometers specialized in semen sexing use the above-described and improved modifications together with modern electronics. This enable to achieve good visualization and separation of the X and Y spermatozoa at the flow of approximately 40,000 cells per second and to obtain about 15-20 million of spermatozoa of fraction purity above 90% within one hour. Such semen is typically frozen in straws, in portions containing 2-2.5 million of spermatozoa. After thawing the semen, the percentage of spermatozoa with progressive movement usually amounts to 50-60%. However, fertilizing potential of the sexed semen of bulls is lower compared to the non-sexed semen, for two reasons. One of them is lower number of spermatozoa in the insemination dose, and the second one is spermatozoa damage that occurs during the sorting process. In the majority of bulls, an important role is played by these both factors, but it has not been established yet which one of them plays the greater role (Seidel, 2014). However, it has been demonstrated that there are great differences in the effectiveness of insemination between bulls after using low doses of spermatozoa in the sexed semen (DeJarnette et al., 2010; 2011).

Studies on the interactions between semen quality and its fertilizing potential are conducted with the use of more and more technically advanced methods. In the previous studies, the following parameters were assessed: spermatozoa viability (Gillan et al., 2008; Christensen et al., 2011), cell membrane integrity (Oliveira et al., 2013; Ahmed et al., 2016), capacitation (Alm et al., 2001; Gillan et al., 2008), acrosome status (Christensen et al., 2011), mitochondrial activity (Ahmed et al., 2016), IVF (Ward et al., 2001), mucus penetration ability (Al Naib et al., 2011), level of reactive oxygen species (Sellem et al., 2015) and chromatin/DNA integrity (Gillan et al., 2008; Ahmed et al., 2016). In spite of the fact that many of these studies were correlated with fertility, there is not an *in vitro* assessed individual feature that will be able to predict fertility reliably enough. This indicates the necessity of introducing a multivariate approach to this issue. It is confirmed in the studies by Sellem et al. (2015) who demonstrated that a combination of computer-aided analysis of spermatozoa movement and cytometric assessment (viability, condition of chromatin and acrosomes, oxidative stress, mitochondrial activity) may better explain (however, in this case only in 40%) differences in fertility. Thus, it is obvious that additional studies considering new quality parameters are required. Recent results of studies (Holden et al., 2017) indicate that the assessment of fertilizing potential of the sexed semen may require the application of other markers than in case of the non-sexed one. Apoptotic markers that were correlated with fertilizing potential of the sexed semen *in vitro* may be useful in this case (Zhao et al., 2014). The application of chemiluminescence methods whose sensitivity is

significantly higher compared to commonly used fluorescent methods seems promising as well (Gogol et al., 2009).

Character of damages of sexed spermatozoa has not been explained enough yet. There are many assumptions, including a possibility of spermatozoa tail stretching in a drop that is formed in nozzle opening or slowing down the progression of the first cell cycle between the fertilization and the first cell division, as a result of permanent binding of Hoechst 33342 dye with sperm (Seidel, 2012). The previous studies indicate a decreased spermatozoa motility after sexing, damages within cell membrane and acrosome and lack of chromatin damage (Boe-Hansen et al., 2005; Suh et al., 2005; Mocé et al., 2006; Carvalho et al., 2010). Unfortunately, the effect of spermatozoa damages on fertility may be only partially compensated by increasing their number in the insemination dose (DeJarnette et al., 2010, 2011). In herds that are properly managed, the application of a dose of 2 million spermatozoa usually results in a fertility at the level of 75-85% compared to control group inseminated with a standard dose of non-sexed semen. Due to the above, the highest cost paid by a breeder as a result of using sexed semen is lower fertility.

The method of sex regulation by dividing the X and Y spermatozoa has become widely used in cattle breeding. Despite higher costs of purchasing sexed semen and its lower fertility, breeding and economic profits that result from obtaining calves of the desired sex are high enough to render this technology profitable. It should be expected that in the following years sexed bull semen will become more widely used in Poland as well.

References

Ahmed H., Andrabi S.M., Jahan S. (2016). Semen quality parameters as fertility predictors of water buffalo bull spermatozoa during low-breeding season. Theriogenology, 86 (6): 1516-1522.

Al Naib A., Hanrahan J.P., Lonergan P., Fair S. (2011). *In vitro* assessment of sperm from bulls of high and low field fertility. Theriogenology, 76: 161-167.

Alm K., Taponen J., Dahlbom M., Tuunainen E., Koskinen E., Andersson M.A. (2001). Novel automated fluorometric assay to evaluate sperm viability and fertility in dairy bulls. Theriogenology, 56: 677-684.

Boe-Hansen G.B., Morris I.D., Ersboll A.K., Greve T., Christensen P. (2005). DNA integrity in sexed bull sperm assessed by neutral comet assay and sperm chromatin structure assay. Theriogenology, 63: 1789-1802.

Carvalho J.O., Sartoric R., Machadoa G.M., Mourãoc G.B., Dode M.A.N. (2010). Quality assessment of bovine cryopreserved sperm after sexing by flow cytometry and their use in *in vitro* embryo production. Theriogenology, 74: 1521-1530.

Christensen P., Labouriau R., Birck A., Boe-Hansen G.B., Pedersen J., Borchersen S. (2011). Relationship among seminal quality measures and field fertility of young dairy bulls using low-dose inseminations. J. Dairy Sci., 94: 1744-1754.

DeJarnette J.M., McCleary C.R., Leach M.A., Moreno J.F., Nebel R.L., Marshall C.E. (2010). Effects of 2.1 and $3.5 \times 10_6$ sex-sorted sperm dosages on conception rates of Holstein cows and heifers. J. Dairy Sci., 93: 4079-4085.

DeJarnette J.M., Leach M.A., Nebel R.L., Marshall C.E., McCleary C.R., Moreno J.F. (2011). Effects of sex sorting and sperm dosage on conception rates in Holstein heifers. Is comparable fertility of sex-sorted and conventional semen plausible? J. Dairy Sci., 94: 3477-3483.

G i l l a n L., K r o e t s c h T., C h i s M a x w e l l W.M., E v a n s G. (2008). Assessment of *in vitro* sperm characteristics in relation to fertility in dairy bulls. Anim. Reprod. Sci., 103: 201-214.

Gogol P., Szczęśniak-Fabiańczyk B., Wierzchoś-Hilczer A. (2009). The photon emission, ATP level and motility of boar spermatozoa during liquid storage. Reprod. Biol., 9 (1): 39-49.

G u t h r i e H.D., J o h n s o n L.A., G a r r e t t W.M., We l c h G.R., D o b r i n s k y J.R. (2002). Flow cytometric sperm sorting: effects of varying laser power on embryo development in swine. Mol. Reprod. Dev., 61: 87-92.

Holden S.A., Fernandez-Fuertes B., Murphy C., Whelan H., O'Gorman A., Brennan L., Butler S.T., Lonergan P., Fair S. (2017). Relationship between *in vitro* sperm functional assessments, seminal plasma composition, and field fertility after AI with either non-sorted or sex-sorted bull semen. Theriogenology, 87: 221-228.

J o h n s o n L.A. (1991). Sex preselection in swine: Altered sex ratios in offspring following surgical insemination of flow sorted X- and Y-bearing sperm. Reprod. Dom. Anim., 26: 309-314.

J o h n s o n L.A., F l o o k J.P., H a w k H.W. (1989). Sex preselection in rabbit: Live births from X and Y sperm separated by DNA and cell sorting. Biol. Reprod., 41: 199-203.

K e e l e r K.D., M a c k e n z i e N.M., Dresser D.W. (1983). Direct microfluorometric analysis of living spermatozoa stained with Hoechst 33342. J. Reprod. Fertil., 68: 205-212.

M o c é E., G r a h a m J.K., S c h e n k J.L. (2006). Effect of sex-sorting on the ability of fresh and cryo-preserved bull sperm to undergo an acrosome reaction. Theriogenology, 66: 929-936.

Oliveira L.Z., de Arruda R.P., de Andrade A.F., Celeghini E.C., Reeb P.D., Martins J.P. (2013). Assessment of *in vitro* sperm characteristics and their importance in the prediction of conception rate in a bovine timed-AI program. Anim. Reprod. Sci., 137: 145-155.

Pinkel D., Lake S., Gledhill B.L., Van Dilla M.A., Stephenson D., Watchmaker G. (1982). High resolution DNA content measurements of mammalian sperm. Cytometry, 3: 1-9.

S e i d e l G.E. Jr. (2012). Sexing mammalian sperm – where do we go from here? J. Reprod. Dev., 58: 505-509.

S e i d e l G.E. Jr. (2014). Update on sexed semen technology in cattle. Animal, 8: 160-164.

Sellem E., Broekhuijse M.L.W.J., Chevrier L., Camugli S., Schmitt E., Schibler L., Koenen E.P.C. (2015). Use of combinations of *in vitro* quality assessments to predict fertility of bovine semen. Theriogenology, 84 (9): 1447-1454.

S m o r ą g Z., R y ń s k a B., K ą t s k a L., S ł o t a E. (1993). Fertilizability of bull spermatozoa stained for flow cytometry. Anim. Sci. Pap. Rep., 11: 117-120.

S u h T.K., S c h e n k J.L., S e i d e l G.E. Jr. (2005). High pressure flow cytometric sorting damages sperm. Theriogenology, 64: 1035-1048.

Ward F., Rizos D., Corridan D., Quinn K., Boland M., Lonergan P. (2001). Paternal influence on the time of first embryonic cleavage post insemination and the implications for subsequent bovine embryo development *in vitro* and fertility *in vivo*. Mol. Reprod. Dev., 60: 47-55.

Z h a o X.M., R e n J.J., Z h a o S.J., C u i L.S., H a o H.S., W a n g H.Y., D u W.H., Q i n T., L i u Y., W a n g D., Z h u H.B. (2014). Apoptosis-like events and *in vitro* fertilization capacity of sexorted bovine sperm. Reprod. Domest. Anim., 49 (4): 543-549.

Accepted for print on 7 August 2018

PIOTR GOGOL

Sexed semen. Reasons for reduced fertility and the possibility of accurate quality assessment

SUMMARY

The use of sexed semen is highly attractive for cattle breeders who want to considerably increase the number of female calves born in a herd. Today, the proportion of female calves obtained after insemination with X-bearing sexed semen is around 90%. Semen-sexing technology is subject to continuous improvement, but it still offers lower conception rates (fertility) compared to conventional semen. The main reason is reduced quality of spermatozoa, which are exposed to technological stress factors during sexing. Therefore, it is of paramount importance that their *in vitro* quality can be accurately assessed. Standard sperm assessment methods such as motility, morphology and survival have many limitations, and their results correlate with sperm's fertilizing capacity only to a certain extent. For this reason, it is necessary to use modern semen assessment methods to provide a more accurate basis for determining semen fertility *in vivo*.

Key words: sexed semen, fertility, cattle