ASAS-SSR Triennial Reproduction Symposium: The use of natural cycle's follicular dynamic to improve oocyte quality in dairy cows and heifers^{1,2}

Marc André Sirard^{†,3}, François Xavier Grand[‡], Remi Labrecque[‡], Christian Vigneault[‡], and Patrick Blondin[‡]

[†]Centre de recherche en reproduction, développement et santé intergénérationnelle (CRDSI), Département des Sciences Animales, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Québec G1V 0A6, Canada; [‡]Semex Inc., Ste-Madeleine, Québec J0H 1S0, Canada

ABSTRACT: The selection of the best dairy heifers is mainly driven by the genetic value of their parents. The phenotype analysis of cows and of the daughters of bulls has been used to identify the best genetic value for decades before being replaced by genomic selection of individuals that are not yet parents. Because it is possible to predict the future value of an individual by its genetic makeup, it becomes feasible to do it as early as the blastocyst stage and to decide which should be transferred or not. Because we know the genotype of an animal at birth, or even before, it is becoming desirable to reproduce this animal as soon as possible to reduce generation interval and improve selection speed. Nature provides constraints that can be overcome: a single oocyte per cycle and age at puberty. Indeed, it is now possible to super-stimulate the ovary at any age and to start collecting oocytes at 6 mo by trans-vaginal ultrasonography. The challenge becomes the production of good eggs and embryos capable of implanting and developing into healthy calves.

Our understanding of ovarian follicular physiology has been instrumental in designing stimulation protocols that may be adjusted to any physiological context including age, and even the individual animal, to obtain a good response. Therefore, the combination of procedures developed in cows to optimize oocyte quality, for example, FSH coasting, in association with in vitro fertilization and optimal culture conditions can now result in the production of several female embryos twice a month from animals 6 to 12 mo of age. The transcriptomic and epigenetic analyses of embryos produced from the same females at different ages were compared and few differences were noted in particular in relation to embryo metabolism. These embryos are as good as the ones obtained from adult animals and can be produced with sexed sperm of bulls 12 mo of age. This combination of these technical optimizations with blastocyst genotyping allows the selection of a second generation within a year.

Key words: reproduction, dairy cow, ovarian follicle, oocyte quality

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

J. Anim. Sci. 2018.96:2971–2976
doi: 10.1093/jas/sky050

¹Based on a presentation entitled “Prime Eggs from Prime Cows,” presented at the ASAS-SSR Triennial Reproductive Symposium, July 13, 2017, Washington, D.C.

²Financial support for this research was provided by the Natural Sciences and Engineering Research Council of Canada – Collaborative Research and Development (NSERC-CRD grant no. RDCPJ461697-13). The authors declare no potential conflict of interests.

³Corresponding author: Marc-Andre.Sirard@fsaa.ulaval.ca

Received December 18, 2017.

Accepted February 23, 2018.

INTRODUCTION

Genetic progress in dairy cattle has been impressive over the last century and the recent use of genomic selection generates even more pressure on breeders to use the optimal combination of genes from each parent to obtain animals with the most valuable characteristics. These traits include low heritability factors such as general health and reproduction efficiency, in addition to production

traits that are still desirable, but proportionally not valued as much as before. Such genetic–economic context increases the demand for the best genetic makeup and the use of younger and younger animals to shorten the generation interval. Puberty can hardly be advanced for physiological reasons in bulls, the use of prepubertal heifers, however, is less restricted as cow ovaries begin the production of follicles, and therefore of eggs, before birth as in most large mammals. Early experiments showed that calves only a few weeks old can be stimulated with exogenous hormones to produce mature oocytes and live offspring (Armstrong et al., 1992). Subsequently, others demonstrated the potential of accessing ovaries at different ages before puberty to obtain an F1 with less invasive approaches such as ultrasound-guided follicular aspiration (Khatir et al., 1998; Majerus et al., 1999). These earlier studies reported that calf oocytes are different (Lévesque and Sirard, 1994; Gandolfi et al., 1998), but can be rescued if provided with the cytoplasm of oocytes from adult animals (Salamone et al., 2001). The understanding of the nature of cytoplasmic maturation progressively changed the methods to control ovarian stimulation to take advantage of the effect of time (Blondin et al., 2002; Sirard et al., 2006). The success rate continued to improve and we can now observe quite remarkable embryo rates following ovarian pick up (OPU), in vitro fertilization (IVF), and in vitro culture from animals as early as 8 mo of age (Landry et al., 2016; Morin-Doré et al., 2017). Such improvement in the quality of embryos generated using pre-pubertal animals comes from a better understanding of the follicular dynamics and the basic physiological events taking place during the last few days of folliculogenesis. The following text summarizes the journey taken to improve egg quality to allow prime animals to create a new generation before reaching puberty.

THE CONCEPT OF FOLLICULAR COASTING

In a natural estrous cycle, the rise of FSH at the beginning of each follicular wave is followed by the recruitment of a dominant follicle within a few days which increases the negative feedback through rising inhibin and estradiol concentrations (Beg and Ginther, 2006). The dominant follicle survives this decrease in FSH by developing sensitive LH receptors that maintain growth and prevent atresia while subordinate follicles stop growing and progressively enter the atresia process within 1 to 2 d (Fortune et al., 2001). This period of time during

folliculogenesis seems important to prepare granulosa cells for the major change that will occur at ovulation: the transformation from an epithelial cell type into a mesenchymal cell type within the future corpus luteum. If basal LH is removed by using a GnRH antagonist, a different gene expression pattern will emerge (Sirard, 2016), and oocyte quality will decrease rapidly as in atretic follicles (Nivet et al., 2016). Other experiments testing the quality of oocytes also confirmed the importance of follicular size to generate blastocysts. However, the effect of a state of early atresia or plateau phase seems more important than follicular size for preparing the oocyte to develop post-fertilization (Blondin and Sirard, 1995; Nivet et al., 2012). The combination of these 2 sources of information, the different follicular conditions under basal LH growth and the beneficial effect of the plateau phase in antral follicles, was instrumental in understanding the importance of reducing FSH for a defined period before harvesting oocytes. It seems that as long as FSH is driving the growth of the follicle, the oocyte does not reduce its transcriptional activity (see below) and does not begin the final preparation leading to ovulation. It is only when FSH decreases that the oocyte will either be programmed by a dominant follicle under basal LH or will start a pseudo-chromatin compaction leading to cell death and resorption of the follicle which happens in most cases in large mammals ovulating only 1 follicle per cycle (Dieci et al., 2016). We now have molecular evidence that during the basal LH period, there is a synchrony between the 3 inner follicle components: the oocyte, the cumulus, and granulosa cells. This synchrony results in the activation of the 5 principal components of differentiation: estradiol dependant genes, *TGFBI*, *TP53*, retinoic acid dependant genes, and *HNF4*, and this activation is instrumental in promoting the changes leading to the epithelial–mesenchymal transition (Khan et al., 2016). The value of these last few days of differentiation was demonstrated in a study where follicles were maintained in the growth phase with FSH and not allowed to go through this low-FSH period. This resulted in a markedly reduced blastocyst rate (Blondin et al., 1996). Accordingly, in the early years of this century, our laboratory designed a FSH withdrawal period to improve the quality of oocytes recovered by OPU and fertilized in vitro (Blondin et al., 2002). After several years of experimenting with the concept, the optimal low FSH period was determined to be 48 to 62 h in adult animals, and the average rate of blastocysts obtained reached 75%, with some animals producing 100%

blastocysts with their complete cohort of follicles (Nivet et al., 2012). Since then, thousands of calves have been produced using this approach which now allows the full potential of Assisted Reproduction Technologies (ART) to be developed in cattle.

THE PHENOMENON OF CHROMATIN PREPARATION AND CONDENSATION

This second piece of the puzzle was provided by the group of Alberto Luciano who made the observation that immature oocytes (germinal vesicle [GV] stage) displayed different chromatin configurations. In very small follicles, the chromatin is diffuse and associated with active transcription (Fair et al., 1997) as the oocyte is still accumulating transcripts to support the transcriptional arrest of 7 d starting in the preovulatory follicle and lasting up to the 8-cell stage in the bovine embryo (Sirard, 2010). As the follicle continues its growth, the chromatin starts to prepare and becomes more compact in the GV-1 type. The chromatin then makes clusters in GV-2 and finally becomes a very dense structure in GV-3 oocytes (see Figure 1; Lodde et al., 2007). These changes are associated with the decrease in transcription and also involve several changes in the histones themselves (Labrecque et al., 2016; Lodde et al., 2017) and their post-translational modifications (Luciano et al., 2012). This transformation is similar, but somewhat different from the change from the nonsurrounded nucleolus pattern to the more compact form surrounded nucleolus as the mouse oocyte acquires developmental competence once it reaches its full size (for a review see Luciano et al., 2014). The other main difference with the mouse seems to be the observation that follicle size in cows does not predict the GV status of the oocyte. Indeed, the distribution of GV-1-2-3 is equal in the different categories of follicle size and corresponds to the growing, plateau, and atretic

phases of follicle development (Dieci et al., 2016). In cows, follicle growth occurs in waves which are modulated by the cycle of a FSH rise followed by the selection of a dominant follicle which lasts a few days with or without ovulation. This leads to a new rise in FSH and this goes on from birth to the end of life including pregnancy periods (Beg and Ginther, 2006).

The progressive change in chromatin configuration is not essential for meiotic resumption as GV-1 oocytes are fully capable of reaching metaphase II in culture; however, developmental competence acquisition (Blondin et al., 2002) is not yet completed indicating that other changes must occur to the chromatin itself or to other structures in the same period. In addition to the histone changes mentioned above, the final methylation of imprinted genes is potentially incomplete if we extrapolate data from the mouse (Hanna and Kelsey, 2017) and make a comparison with the surrounded nucleolus configuration. In the mouse, several imprinted genes (*Igf2*, *Mest*, *Snrpn*, *Kcnq1*, *Peg3*, *Zac1*, *Nespas*, *Socs5*, and *Igf2r*) are only fully programmed in the last growth period before the oocyte reaches its full size (60 to 65 μ m). The cytoplasmic changes occurring during oocyte meiotic resumption have not been reported to be affected by the GV status; however, the ultrastructure of mature or maturing oocytes has never been correlated with their initial GV status at aspiration. Transcriptome analysis of oocytes according to GV status revealed numerous histone variant changes (Landry et al., 2016), as well as hundreds of other modifications in the RNA content associated with genes that are either stored (Gohin et al., 2014) or translated during the maturation period (Scantland et al., 2014). The difficulty with RNA analysis is that oocytes store numerous transcripts through deadenylation (leaving around 25 As), and the amplification systems that are used in molecular biology

Figure 1. The variation of chromatin configuration during follicular coasting in prepubertal dairy heifers using Hoechst staining at the GV stage immediately following ovarian pick up. GV1 status, GV2 status, and GV3 status are represented.

normally prime on the poly A tail and do not differentiate the short (stored) vs. the long poly A tails (> 100 As) that are rapidly translated, thus creating a doubt as to whether the transcripts are used during the transition between GV stages or stored for maturation or post-fertilization events. Clearly, the process is dynamic and the oocyte content is modified as ovulation gets closer. Surprisingly, the changes leading to atresia and ovulation are partly similar, both in the gene expression profiles and in the microscopic observation of the chromatin compactness during these 2 events (Dieci et al., 2016).

THE CONCEPT OF “BEST BEFORE 60 H” FOR OOCYTE QUALITY

In a natural bovine estrous cycle, the period between the drop in FSH and ovulation varies between 4 and 5 d. During this period, as mentioned above, basal LH maintains the growth of the follicle but also induces another type of differentiation compared with the FSH response. The comparative analysis of gene expression from the FSH growth phase indicates that cell multiplication is reduced during the plateau to permit progressive differentiation leading to cell secretion (e.g., estradiol) and accumulation of follicular fluid to generate volume faster than tissue growth (Nivet et al., 2012; Douville and Sirard, 2014; Girard et al., 2015). This plateau phase, which is characterized by follicle size increase with low cell division and fluid accumulation, seems to have a limited duration. In bovine, the dominant follicle will regress if progesterone remains high creating waves of follicular emergence (2 or 3 per cycle; Beg and Ginther, 2006). The reason why cow follicles have a limited lifespan is not completely clear. In other species like humans, the dominant follicle normally goes to ovulation at each cycle. Nevertheless, using hormonal stimulation and antagonist blockers, the more advanced follicles in humans also contain oocytes of reduced quality (Nivet et al., 2016). The possible explanation for the reduced lifespan is the oocyte transcriptional arrest that occurs when chromatin compaction is completed as described above. The ability of the oocyte to maintain homeostasis is necessarily limited with a marked reduction of the capacity to make new proteins from new RNAs, and at the same time, the duration of the estradiol response of the uterus may not last indefinitely if no ovulation occurs.

This phenomenon of chromatin compaction and limited lifespan of the oocyte is not often considered in super-stimulation treatments. When cows

are stimulated for embryo flushing at day 7, FSH is used to increase the number of growing follicles; and although the removal of any dominant follicles 2 d before the beginning of FSH treatment results in better synchrony of growth, the follicular distribution remains large. When prostaglandins are then used to trigger corpus luteum decay and ovulation, a large range of follicular sizes remains, and rarely do 100% of ovulations result in day-7 embryos. Although the time between FSH arrest and ovulation is shorter (2 to 3 d) than the natural ovulation cycle, it seems sufficient for most follicles to go through the transformation leading to a mature oocyte and the formation of a corpus luteum. When stimulation with FSH is used prior to OPU to obtain immature oocytes, the process of creating a plateau phase, where basal LH induces the required changes in follicles and oocyte differentiation, becomes important. Indeed, we showed in the work of Blondin et al. (1996) that actively growing follicles contain less competent oocytes than early atretic ones. This concept of early atresia benefit has been extended to other species and refined for using OPU on nontreated animals by creating the right amount of time for a follicular wave to reach the early dominance before collection. In the stimulation context where all the follicles of a wave become dominant (acquire LH receptors), the mimicking of the low FSH/basal LH situation becomes important to increase oocyte quality. Time line studies comparing each animal to itself and using 1, 2, 3, or 4 d of coasting under inhibitory concentrations of progesterone showed an increase followed by a decrease in oocyte quality as measured by blastocyst rates. The optimal coasting time was set at 44 to 72 h for most adult

Figure 2. The rise and drop of FSH 4 d before ovulation. The follicular growth phase is mainly under the influence of FSH and the follicular differentiation phase is supported by basal LH until the LH surge and this period lasts 4 d.

animals indicating a limited window of opportunity to achieve the best results (Figure 2; Nivet et al., 2012). This period of average 60 h is similar to the time that normally elapses between the natural decrease of FSH and ovulation. This coasting protocol is increasingly used in dairy cows where the cost of the procedure is justified by the value of the embryos and where sexing the semen results is a real economic advantage.

USING THE ABOVE CONCEPT TO IMPROVE OOCYTE QUALITY FROM PREPUBERTAL ANIMALS

The genomic pressure to select animals with the right genetic combination either to generate new bulls or to breed even better cows has pushed the development of ART to include prepubertal animals. Although it was shown more than 25 yr ago that calf oocytes could be fertilized and produce live births, the process has been limited by the low success rate and the invasive laparoscopic procedure. The focus on animals where ultrasound-guided oocyte recovery is possible (>6 mo of age) resulted in improved success rates especially using the coasting concept described above (Landry et al., 2016). Indeed, with the right window of FSH withdrawal, blastocyst rates can be as high as or slightly lower than the ones obtained with oocytes from adult animals. Basal LH in calves is not very well studied, but follicle growth is observed from before birth to puberty, and such growth is believed to require a minimal amount of LH to support theca cell functions. It is possible that in some animals, basal LH would be insufficient to maintain growth of dominant follicles, explaining a faster turnover and the less efficient coasting compared with adults. Nevertheless, the results are very good with animals from 8 mo of age (Landry et al., 2017), and progress is being made with animals 6 to 8 mo of age to achieve the same success as with adult animals.

The next limit would be to start at 4 to 5 wk of age, but because the ovaries are not firmly fixed in the abdomen, ultrasound does not work very well without transrectal manipulation of the ovaries. Laparoscopic recovery is possible, and has been done, but is more problematic than ultrasound as it requires a surgery setting and may leave adhesions if used repeatedly on the same animal (Sirard and Lambert, 1986).

CONCLUSION

The progressive understanding of the final steps in the timing of bovine folliculogenesis is now

permitting the recovery of healthy oocytes from prepubertal dairy heifers. This achievement may improve the acceleration of genetic gain, as the generation interval is decreased substantially.

LITERATURE CITED

- Armstrong, D. T., P. Holm, B. Irvine, B. A. Petersen, R. B. Stubbings, D. McLean, G. Stevens, and R. F. Seamark. 1992. Pregnancies and live birth from in vitro fertilization of calf oocytes collected by laparoscopic follicular aspiration. *Theriogenology*. 38:667–678.
- Beg, M. A., and O. J. Ginther. 2006. Follicle selection in cattle and horses: role of intrafollicular factors. *Reproduction* 132:365–377. doi:10.1530/rep.1.01233.
- Blondin, P., D. Bousquet, H. Twagiramungu, F. Barnes, and M. A. Sirard. 2002. Manipulation of follicular development to produce developmentally competent bovine oocytes. *Biol. Reprod.* 66:38–43.
- Blondin, P., M. Dufour, and M. A. Sirard. 1996. Analysis of atresia in bovine follicles using different methods: flow cytometry, enzyme-linked immunosorbent assay, and classic histology. *Biol. Reprod.* 54:631–637.
- Blondin, P., and M. A. Sirard. 1995. Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. *Mol. Reprod. Dev.* 41:54–62. doi:10.1002/mrd.1080410109.
- Dieci, C., V. Lodde, R. Labreque, I. Dufort, I. Tessaro, M. A. Sirard, and A. M. Luciano. 2016. Differences in cumulus cell gene expression indicate the benefit of a pre-maturation step to improve in-vitro bovine embryo production. *Mol. Hum. Reprod.* 22:882–897. doi:10.1093/molehr/gaw055.
- Douville, G., and M. A. Sirard. 2014. Changes in granulosa cells gene expression associated with growth, plateau and atretic phases in medium bovine follicles. *J. Ovarian Res.* 7:50. doi:10.1186/1757-2215-7-50.
- Fair, T., S. C. Hulshof, P. Hyttel, T. Greve, and M. Boland. 1997. Nucleus ultrastructure and transcriptional activity of bovine oocytes in preantral and early antral follicles. *Mol. Reprod. Dev.* 46:208–215. doi:10.1002/(SICI)1098-2795(199702)46:2<208::AID-MRD11>3.0.CO;2-X.
- Fortune, J. E., G. M. Rivera, A. C. Evans, and A. M. Turzillo. 2001. Differentiation of dominant versus subordinate follicles in cattle. *Biol. Reprod.* 65:648–654.
- Gandolfi, F., E. Milanese, P. Pocar, A. M. Luciano, T. A. Brevini, F. Acocella, A. Lauria, and D. T. Armstrong. 1998. Comparative analysis of calf and cow oocytes during in vitro maturation. *Mol. Reprod. Dev.* 49:168–175. doi:10.1002/(SICI)1098-2795(199802)49:2<168::AID-MRD7>3.0.CO;2-N.
- Girard, A., I. Dufort, G. Douville, and M. A. Sirard. 2015. Global gene expression in granulosa cells of growing, plateau and atretic dominant follicles in cattle. *Reprod. Biol. Endocrinol.* 13:17. doi:10.1186/s12958-015-0010-7.
- Gohin, M., E. Fournier, I. Dufort, and M. A. Sirard. 2014. Discovery, identification and sequence analysis of rnas selected for very short or long poly a tail in immature bovine oocytes. *Mol. Hum. Reprod.* 20:127–138. doi:10.1093/molehr/gat080.
- Hanna, C. W., and G. Kelsey. 2017. Genomic imprinting beyond dna methylation: a role for maternal histones. *Genome Biol.* 18:177. doi:10.1186/s13059-017-1317-9.

- Khan, M. I., F. C. Dias, I. Dufort, V. Misra, M. A. Sirard, and J. Singh. 2016. Stable reference genes in granulosa cells of bovine dominant follicles during follicular growth, fsh stimulation and maternal aging. *Reprod. Fertil. Dev.* 28:795–805. doi:10.1071/RD14089.
- Khatir, H., P. Lonergan, J. L. Touzé, and P. Mermillod. 1998. The characterization of bovine embryos obtained from prepubertal calf oocytes and their viability after non surgical embryo transfer. *Theriogenology* 50:1201–1210.
- Labrecque, R., E. Fournier, and M. A. Sirard. 2016. Transcriptome analysis of bovine oocytes from distinct follicle sizes: insights from correlation network analysis. *Mol. Reprod. Dev.* 83:558–569. doi:10.1002/mrd.22651.
- Landry, D. A., A. M. Bellefleur, R. Labrecque, F. X. Grand, C. Vigneault, P. Blondin, and M. A. Sirard. 2016. Effect of cow age on the in vitro developmental competence of oocytes obtained after fsh stimulation and coasting treatments. *Theriogenology* 86:1240–1246. doi:10.1016/j.theriogenology.2016.04.064.
- Landry, D. A., C. Fortin, A. M. Bellefleur, R. Labrecque, F. X. Grand, C. Vigneault, P. Blondin, and M. A. Sirard. 2017. Comparative analysis of granulosa cell gene expression in association with oocyte competence in fsh-stimulated holstein cows. *Reprod. Fertil. Dev.* 29:2324–2335. doi:10.1071/RD16459.
- Lévesque, J. T., and M. A. Sirard. 1994. Proteins in oocytes from calves and adult cows before maturation: relationship with their development capacity. *Reprod. Nutr. Dev.* 34:133–139.
- Lodde, V., A. M. Luciano, F. Franciosi, R. Labrecque, and M. A. Sirard. 2017. Accumulation of chromatin remodelling enzyme and histone transcripts in bovine oocytes. *Results Probl. Cell Differ.* 63:223–255. doi:10.1007/978-3-319-60855-6_11.
- Lodde, V., S. Modina, C. Galbusera, F. Franciosi, and A. M. Luciano. 2007. Large-scale chromatin remodeling in germinal vesicle bovine oocytes: interplay with gap junction functionality and developmental competence. *Mol. Reprod. Dev.* 74:740–749. doi:10.1002/mrd.20639.
- Luciano, A. M., F. Franciosi, C. Dieci, and V. Lodde. 2014. Changes in large-scale chromatin structure and function during oogenesis: a journey in company with follicular cells. *Anim. Reprod. Sci.* 149:3–10. doi:10.1016/j.anireprosci.2014.06.026.
- Luciano, A. M., V. Lodde, F. Franciosi, I. Tessaro, D. Corbani, and S. Modina. 2012. Large-scale chromatin morpho-functional changes during mammalian oocyte growth and differentiation. *Eur. j. Histochem.* 56:e37. doi:10.4081/ejh.2012.e37.
- Majerus, V., R. De Roover, D. Etienne, S. Kaidi, A. Massip, F. Dessy, and I. Donnay. 1999. Embryo production by ovum pick up in unstimulated calves before and after puberty. *Theriogenology* 52:1169–1179. doi:10.1016/S0093-691X(99)00209-5.
- Morin-Doré, L., P. Blondin, C. Vigneault, F. X. Grand, R. Labrecque, and M. A. Sirard. 2017. Transcriptomic evaluation of bovine blastocysts obtained from peri-pubertal oocyte donors. *Theriogenology* 93:111–123. doi:10.1016/j.theriogenology.2017.01.005.
- Nivet, A. L., A. Bunel, R. Labrecque, J. Belanger, C. Vigneault, P. Blondin, and M. A. Sirard. 2012. fsh withdrawal improves developmental competence of oocytes in the bovine model. *Reproduction* 143:165–171. doi:10.1530/REP-11-0391.
- Nivet, A. L., M. C. Léveillé, A. Leader, and M. A. Sirard. 2016. Transcriptional characteristics of different sized follicles in relation to embryo transferability: potential role of hepatocyte growth factor signalling. *Mol. Hum. Reprod.* 22:475–484. doi:10.1093/molehr/gaw029.
- Salamone, D. F., P. Damiani, R. A. Fissore, J. M. Robl, and R. T. Duby. 2001. Biochemical and developmental evidence that ooplasmic maturation of prepubertal bovine oocytes is compromised. *Biol. Reprod.* 64:1761–1768.
- Scantland, S., I. Tessaro, C. H. Macabelli, A. D. Macaulay, G. Cagnone, É. Fournier, A. M. Luciano, and C. Robert. 2014. The adenosine salvage pathway as an alternative to mitochondrial production of atp in maturing mammalian oocytes. *Biol. Reprod.* 91:75. doi:10.1095/biolreprod.114.120931.
- Sirard, M. A. 2010. Activation of the embryonic genome. *Soc. Reprod. Fertil. Suppl.* 67:145–158.
- Sirard, M. A. 2016. Somatic environment and germinal differentiation in antral follicle: the effect of FSH withdrawal and basal LH on oocyte competence acquisition in cattle. *Theriogenology* 86:54–61. doi:10.1016/j.theriogenology.2016.04.018.
- Sirard, M. A., and R. D. Lambert. 1986. Birth of calves after in vitro fertilisation using laparoscopy and rabbit oviduct incubation of zygotes. *Vet. Rec.* 119:167–169.
- Sirard, M. A., F. Richard, P. Blondin, and C. Robert. 2006. Contribution of the oocyte to embryo quality. *Theriogenology* 65:126–136. doi:10.1016/j.theriogenology.2005.09.020.