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Cellular and molecular mechanisms regulating oocyte quality and the relevance for farm animal reproductive efficiency

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Summary

The efficiency of breeding schemes is dependant on the high fecundity of the selected individuals. Reproductive technologies are constantly pushing the physiological limits, but while the male reproductive potential is almost fully exploited, female reproductive physiology is the subject of constant research. Since the number of offspring that a female can bring to term each pregnancy cannot be changed, the ideal approach is to remove the potential offspring at the beginning of development and to transfer them to recipients of lesser genetic value. The earlier the collection takes place, the higher the number of descendants that a female can generate, so that now, the number of available oocytes becomes the limiting factor. This article will describe how detailed studies on oocyte physiology are beginning to unravel the complex sequence that transforms a small primordial follicle into a large ovulatory follicle containing a mature oocyte. Progressively, the limits to oocyte manipulation have been recognised and gradually overcome with adequate hormonal treatments *in vivo* and with specific media supplementation *in vitro*. This has lead to the development of highly efficient reproductive technologies and the promise of even greater advances in the future. Surprising new findings, such as ovarian stem cells that can replenish the follicle population or long term embryonic stem cell lines that can differentiate into oocytes, are rapidly changing our expectations.

Keywords

Cyclic adenosine monophosphate – Embryo – Fecundity – Follicle – In vitro fertilisation – Maternal messenger ribonucleic acid – Maturation – Oocyte – Ovary.

Introduction

Farm animal reproduction has long taken advantage of genetic diversity in order to improve breeding schemes, increase fecundity and maximise the number of offspring from a given set of parents. However, while high fecundity is essential for increasing the rate of genetic gain and for the dissemination of desirable genetics throughout the livestock population, excessive inbreeding, which would have long-term negative consequences on the genetic diversity of the population, must be avoided (68).

A wide range of reproductive technologies has been developed over the past few decades, spanning from pioneering techniques, such as artificial insemination, to increasingly sophisticated methods such as embryo transfer, embryo splitting, *in vitro* embryo production, juvenile reproduction, ovum pick-up (OPU) and cloning. Detailed descriptions of these and other methods, including the genetic pros and cons, can be found elsewhere in this issue. This paper will focus on the common limiting factor of these techniques: the availability of fertilisable oocytes. Is it possible to circumvent this obstacle and have an unlimited supply of oocytes, which would allow for a dramatic increase in the performance level of embryo-based reproductive technologies, and, more importantly, will this be compatible with animal health and welfare? This article will discuss the cellular and molecular mechanisms that underlie the availability of fertilisable oocytes and the potential techniques that could be used in the future to circumvent this limitation.

The origin of the oocyte population

Unlike spermatozoa, which are generated continuously from puberty onwards, there is a finite population of oocytes. The total number of oocytes present in the adult ovary originates from a definite number of primordial germ cells (PGCs) that are formed in the yolk sac epithelium of the embryo. These cells reach the primitive ovary after migrating through the gut mesentery and the gonadal ridges of the mesonephros of the early embryo (11). Once PGCs have reached the developing ovary the cells begin to differentiate into oogonia. The population of oogonia proliferates until shortly before birth at which time the oogonia enter meiosis and are termed primary oocytes (29). The process of meiosis will halve the number of chromosomes resulting in the creation of haploid oocytes that are ready for fertilisation by sperm carrying the other half of the genome. However, primary oocytes progress only through part of meiosis and arrest at the dictyate stage (55).

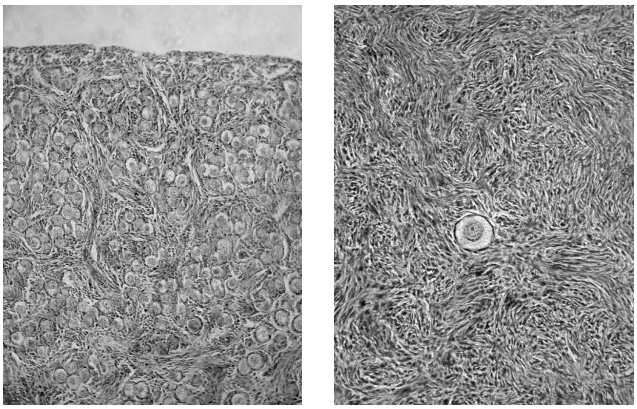
When an oocyte enters meiosis a single layer of flattened pregranulosa cells encloses it, thus forming the primordial follicle. This is the first step of folliculogenesis: the process that leads to the formation and growth of the ovarian follicle. The follicle structure is completed by the appearance of several layers of stromal cells that will differentiate into the theca layers after follicle growth commences (30). Once established, the follicular unit helps to maintain the oocyte in a controlled environment and isolates the cell from any potentially harmful substances circulating in the bloodstream. Oocytes that do not become incorporated into primordial follicles will degenerate. The vast majority of primary oocytes are not incorporated into primordial follicles resulting in a loss of up to 60% in sows (32) and more than 90% in ewes (32) and cows (22) of the original oocyte pool at the time of birth.

Primordial follicles constitute the store of germ cells in the postnatal ovary (Fig. 1a). The number of primordial follicles varies between species (ranging from approximately 500 000 in pigs to 135 000 and 82 000 in cows and ewes, respectively [55]) and with the age of the animal.

As soon as the primordial follicle store is established, follicle recruitment begins and continues without interruption until the animal is slaughtered or until the ovary is depleted, whichever occurs first (Fig. 1b). Cohorts of follicles are selected for growth, and follicular growth occurs in a wave-like pattern during oestrous cycles. This pattern of follicular growth was first established in cattle (57) and has since been shown to occur in most other species. Follicle growth is continuous and in most cases ends with the degeneration (atresia) of the follicle and its oocyte. Once follicles form an antrum and reach a certain diameter (i.e. 4 mm-6 mm in cattle and 2 mm-4 mm in sheep) the growth of the follicle becomes gonadotrophin dependent (18). When a follicular wave is exposed to the adequate hormonal milieu, selection takes place and the number of growing follicles in a wave is reduced to a predefined number that will ovulate in a given species. Among domestic species, pigs are the only species that do not have follicle waves during the oestrous cycle. Waves of follicular development also occur before puberty and during pregnancy, but in these circumstances ovulation does not occur because hormonal levels are not appropriate (23).

The number of oocytes that reach ovulation is obviously limited to the number of offspring that each species can bring to term in the uterus. From the several thousand primary oocytes available at birth, the number of oocytes that will be fertilised and develop to term following natural mating or artificial insemination is reduced to only a few. Reproductive technologies initially overcame this limit by collecting pre-implantation embryos from valuable donor animals that were superovulated and transferring them to recipient animals. It was soon evident that collecting oocytes instead of embryos would increase the efficiency of this approach. Moreover, the further away from the time of ovulation the collection takes place, the greater the number of available oocytes (50). In theory, the earlier the collection takes place (i.e. at birth, or even before birth, when the pool of several thousand primordial follicles can be exploited), the greater the number of oocytes with the potential to be fertilised and developed to term.

The question is, what is the limit to which technology can be pushed before the health of the donor and, more importantly, of the offspring is compromised?



a) original magnification 200 × b) original magnification 400 ×

Fig. 1

Primordial follicles observed in the ovary: appearance of the ovarian cortex in (a) a newborn (b) an aged animal

The role of oocyte quality

Increasing the number of fertilisable oocytes by removing them from the ovary before ovulation has consequences. Large field trials performed under commercial conditions have shown that pregnancy rates after transfer of fresh or frozen-thawed *in vitro*-produced embryos are significantly reduced compared to the rates achieved with *in vivo* produced counterparts (28, 34, 72). *In vitro* embryo production consists of three phases: oocyte maturation, *in vitro* fertilisation and embryo culture. A number of reports in the literature indicate that oocytes matured *in vivo* are more competent than those matured *in vitro*, thus highlighting the crucial role of oocyte maturation (6, 31, 43, 49, 60, 70, 71).

However, oocytes are not all equal. In cattle, follicles with a large diameter have been shown to contain oocytes with higher developmental potential (45). Oocyte diameter is directly proportional to follicle diameter, and the oocyte continues to grow even in follicles with a diameter > 10 mm (2). Therefore, follicle size and oocyte diameter are closely related, and as both increase the developmental capability of the oocytes improves (Fig. 2).

This indicates that oocyte competence is acquired within the ovary during the developmental stages that precede ovulation through a process referred to as 'oocyte capacitation' (38). Though the precise mechanisms are unclear, it can be hypothesised that during capacitation oocytes become equipped for future embryonic development, and during maturation an appropriate signal must be provided in order to trigger the developmental programme acquired during capacitation (51).

This is consistent with the observation that oocytes collected from presumptive dominant follicles (> 13 mm) yield a significantly higher blastocyst rate compared to oocytes obtained from follicles of 3 mm to 8 mm (33). However, oocytes isolated from follicles with early signs of atresia also have a high capacity to develop to the blastocyst stage (4), and ultrastructural studies have demonstrated similar cytoplasmic remodelling in oocytes undergoing pre-maturation or early atresia (38).

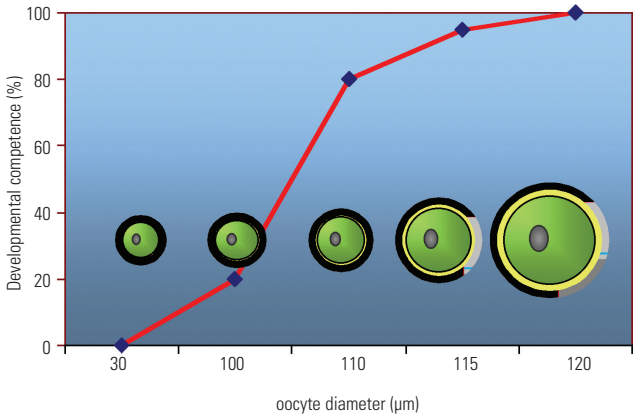


Fig. 2
The relationship between oocyte developmental competence to the blastocyst stage and oocyte diameter
Values are theoretical and pertain to the cow (26)

Applying this knowledge, the administration of follicle stimulating hormone (FSH) followed with ultrasound-guided OPU at 48 hours post-injection, has enabled the harvest of immature oocytes that will almost all develop into embryos (5). The hormonal treatment used in this protocol mimics the natural ovulatory process (i.e. there is an increase in FSH followed by a decrease a few days before ovulation). Unlike the hormonal fluctuations of the natural oestrous cycle, in this case, progesterone does not decrease to allow ovulation, and, therefore, the follicles begin to undergo atresia. However, the appropriate signals have been given to the oocytes so that when the oocytes are removed from the compromised follicle they will be fully competent and capable of development to the blastocyst stage.

Though very effective, hormonal stimulation is not always desirable or possible (e.g. as part of the reproductive technology schemes used to maintain the genetic diversity of rare breeds or endangered species).

The role of the *in vitro* maturation environment

As indicated by the experiments described above, the quality of the follicular environment from which the oocyte originates has a major impact on the quality of the oocyte. During *in vitro* maturation (IVM) the functional unit is not the oocyte alone, but rather the oocyte and the surrounding cumulus cells (referred to as the cumulus-oocyte complex [COC]). Therefore, it is important to take into consideration the nutrient requirements of the COC in order to improve *in vitro* maturation media (65).

Somatic cells (cumulus cells) and germ cells (oocytes) form a functional unit consisting of physical contacts between the cells, mediated by a dense web of gap junctions, and a paracrine signaling system. While, traditionally, communication was believed to occur only between the granulosa cells and the oocytes, an increasing amount of evidence has clearly demonstrated that the communication is actually bidirectional (1). In particular, growth differentiation factor-9 and bone morphogenetic protein 15 (also called GDP-8) play a key role (20) in the communication process.

Communication between the oocyte and the cumulus cells, and between individual cumulus cells, takes place through

specialised membrane channels (called gap junctions) that allow the transfer of low molecular weight molecules (19). The molecules involved in cellular communication include glucose metabolites, amino acids, and nucleotides, but a special role is played by cyclic adenosine monophosphate (cAMP) and purines, which are small regulatory molecules that regulate the oocyte meiotic process at the time of ovulation or *in vitro* maturation (15, 21).

Cyclic AMP acts as the intracellular messenger for gonadotropin stimulation (15). However, the precise mechanism by which changes in the intracellular concentration of cAMP affect oocyte maturation is not fully understood. High levels of cAMP have been proposed as the regulatory mechanism responsible for maintaining the oocyte in meiotic arrest. When oocytes are removed from the follicular compartment and cultured with agents that maintain high intracellular concentrations of cAMP, the oocytes remain in the germinal vesicle (GV) stage. Moreover, membrane-permeable cAMP analogues (e.g. dibutyryl-cAMP, 8-Br-cAMP), forskolin (which activates adenylate cyclase), and the nonselective phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (which prevents cAMP degradation) are all able to inhibit spontaneous oocyte maturation (3, 35). Conversely, transient elevations of cAMP, generated by cAMP analogues or by IBMX, can induce mouse and rat oocyte maturation *in vitro* (16, 17). Similar results were obtained with bovine oocytes in which the intracellular concentration of cAMP was manipulated through the addition of invasive adenylate cyclase (iAC), an enzyme purified from *Bordetella pertussis*, to the IVM medium (47). At the correct dose (0.01 µg/ml) and in the absence of serum and gonadotropins, iAC not only promoted a very high maturation rate, but also significantly improved the rate of blastocyst development. The positive effect was associated with a prolonged gap junction permeability that presumably enhanced the communication between the cumulus cells and the oocyte during IVM (48).

Cyclic AMP is only one of a complex network of paracrine regulators that orchestrate the follicular changes leading to ovulation and full oocyte competence (24). It has been recently demonstrated that amphiregulin, epiregulin, and betacellulin, three growth factors with an epidermal growth factor-like motif, are the physiological mediators of luetinising hormone (LH) stimulation during ovulation (54). All of these findings provide a framework with which to explain the complex effects of FSH and LH on the follicle and the concerted changes taking place in somatic and germ cell compartments: the understanding of which provides a rational basis for improving *in vitro* maturation conditions.

The study of the follicular environment in which oocyte maturation takes place may be useful not only to improve the efficiency of *in vitro* reproductive technologies, but also to explain some of the clinical observations.

It is well known that reproductive efficiency in high yielding dairy cows has decreased over the past few decades. Nutritional changes that have been implemented to achieve the levels of energy and protein intake necessary to support high milk production have been suggested as a possible cause of the reduction in reproductive efficiency. Though the mechanism is not known, much of the data suggest that some form of oocyte damage, possibly mediated by non-esterified fatty acids (NEFA), is occurring during the post-partum period. High concentrations of NEFA have been measured in the blood of high-yielding dairy cows shortly after parturition. These elevated levels can persist for up to three weeks post partum and are caused by a negative energy balance (NEB). This has possible implications for reproductive health because NEFA uptake occurs in the ovary and NEFA concentration in plasma is closely correlated with that in follicular fluid (14, 40, 58). This hypothesis has been recently confirmed *in vitro* by adding NEFA to the IVM medium at doses compatible with the NEB situation observed in post-partum high-yielding dairy cows. Lower percentages of metaphase II (MII) oocytes, a reduced fertilisation rate, and a significant decrease in the rate of cleavage and development to the blastocyst stage were observed (41). However, when nutrition effects are examined in sheep the conclusions are not as clear. Comparisons between sheep fed an ad libitum diet versus those fed a diet with a severely restricted energy content indicated that embryos obtained from superovulated sheep that had been overfed had a lower developmental competence, but it was concluded that this was unlikely to be the result of a direct negative effect of the diet on oocyte quality (46). However, an effect of diet on sheep oocyte quality was observed when energy and urea levels in the diet were manipulated. In this experiment, high levels of energy and urea had a negative effect on the cleavage rate, but no differences were observed in the rate of development to the blastocyst stage (53).

The role of oocyte messenger ribonucleic acid

From the data summarised above it is apparent that the events preceding oocyte collection will have a significant effect later in development. This indicates that the oocyte has developed a mechanism to store the information acquired during its growth and maturation and to use this information at the appropriate time (8).

Information stored in the oocyte is particularly critical during the interval between fertilisation and the so-called maternal-embryonic transition (MET) when transcriptional activity of the embryonic genome becomes fully functional. During this period, embryonic development is supported by maternal ribonucleic acids

(RNAs) and proteins that are synthesised during oogenesis. The length of this period depends on the species. In mammals, the MET can occur as early as the late 2-cell stage, such as in the mouse, or later in development, such as the 4-cell stage in pigs, between the 4-cell and 8-cell stage in human embryos, the 8-cell stage in rabbits, and between the 9-cell and 16-cell stage in sheep and bovine embryos (66).

In cattle oocytes transcriptional activity of the maternal genome has been reported as early as the secondary follicle stage. Synthesis of both heterogeneous nuclear RNA (the precursor of messenger RNA) and ribosomal RNA is initiated during this stage and continues until the oocyte reaches a diameter of 110 µm and is enclosed in a 2 mm to 3 mm follicle (25). This transcriptional activity provides oocyte cytoplasmic stores of messenger molecules, which will be translated until, and possibly beyond (as suggested by evidence obtained in different species), the MET. In the mouse, as much as 30% of maternal messenger RNA (mRNA) is still detectable at the blastocyst stage in both the trophectoderm and the inner cell mass (59). Therefore, the stability of oocyte mRNA is crucial for normal development, and any perturbation of this delicate process is likely to reduce oocyte developmental competence and cause an arrest of embryonic development, which may occur at any given stage.

Various mechanisms have been proposed that describe the storage of mRNAs and the regulation of the expression of mRNAs by the developing oocyte. Information available to date indicates that specific deoxyribonucleic acid (DNA) sequences regulate mRNA stability, control translational activation and repression, and direct mRNA localisation. These sequences are located in the untranslated region of the 3' end of the mRNA molecule. It has been shown that regulation of maternal mRNA translation is based on changes in the length of the poly(A) tail of the mRNAs. Oocyte mRNAs with short poly(A) tails are translationally inactive and are activated upon extension of the tail during specific stages of embryo development (8).

The authors have recently shown that bovine oocytes undergoing meiotic maturation display a temporal regulation of maternal mRNA polyadenylation (7). The use of bovine *in vitro* embryo production as an experimental system has made it possible to correlate developmental competence with polyadenylation patterns. Data obtained from these studies indicate that oocytes that have not yet achieved full competence contain mRNA strands with shorter poly(A) tails than fully competent oocytes; this difference is present at the GV stage and the MII phase (7). These observations indicate a possible relationship between the extent of polyadenylation, mRNA stability, and the state of competence during oocyte maturation. Further analysis has indicated that polyadenylation can be used as marker of developmental competence up to the first embryonic cleavage. A clear relationship exists between the rate of embryo cleavage and specific polyadenylation patterns (9).

Cytoplasmic compartmentalisation has also been suggested as an important factor in the coordination of nuclear and cytoplasmic oocyte maturation (13). The activity and cytoplasmic distribution of mitochondria can be used as a marker of cytoplasm compartmentalisation and is one of the many diverse features of cytoplasmic maturation (69). The primary function of mitochondria is to generate adenosine triphosphate (ATP), which is necessary for several functions, including motility, maintenance of homeostasis, and regulation of cell survival (62). The pattern of distribution and the metabolic activity of mitochondria change during oocyte maturation in many species, including the mouse (12), hamster (61), cow (63) and pig (64). The authors have recently established a relationship between oocyte quality and the 'correct' pattern of cytoplasmic remodelling (10). Fluorescent probes were used to demonstrate that the migration of active mitochondria across the cytoplasm, which is mediated by a microtubule network, during the process of *in vitro* maturation is necessary in order to achieve full developmental competence (Fig. 3).

Conclusions and future directions

As briefly summarised, an understanding of the mechanisms regulating oocyte development and the

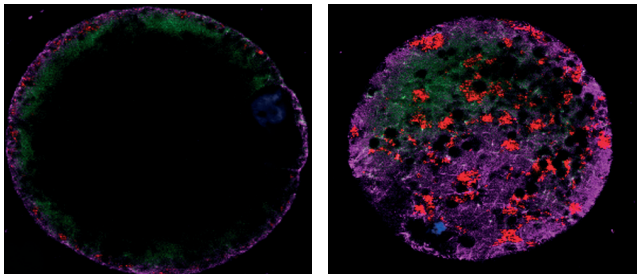


Fig. 3
Pig oocytes stained for mitochondria (red), microtubules (green), kinesines (magenta), and deoxyribonucleic acid (blue)
Oocytes with low developmental competence (left) display a very weak staining that is confined to the cortical area. The uneven distribution will probably cause incorrect compartmentalisation of the ooplasm. Conversely, oocytes with high developmental competence (right) show a uniform distribution of cytoplasmic organelles with a homogeneous localisation of the mitochondria throughout the ooplasm (original magnification 400 ×).

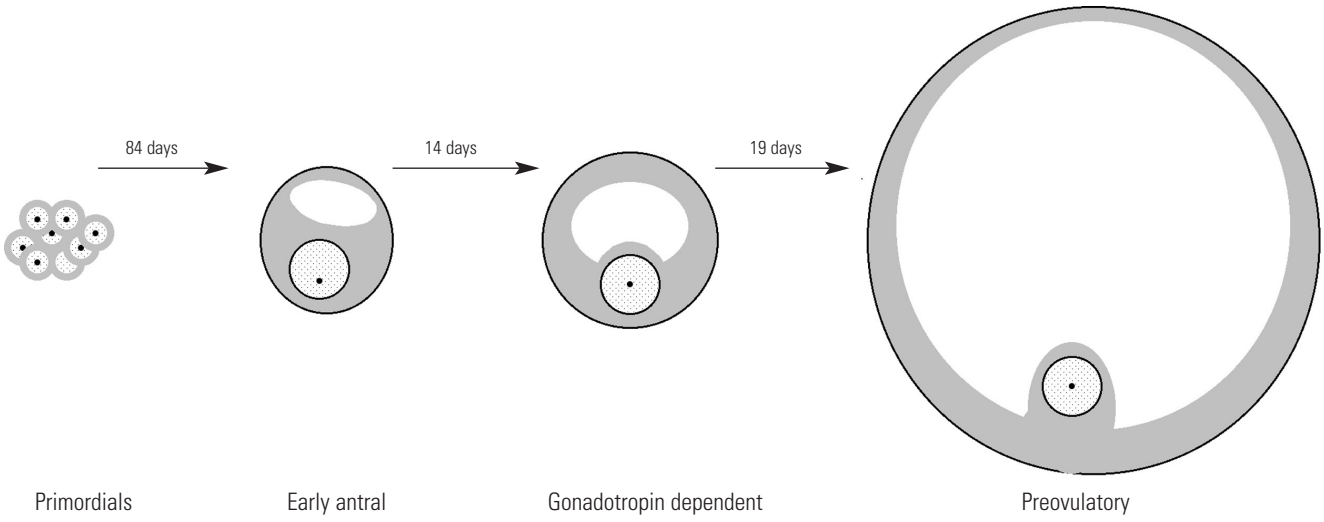


Fig. 4
***In vitro* development of prenatal follicles in farm animals is made difficult by the extended length of time required for full follicle development during which degenerative changes may take place**
Values indicated pertain to the pig (37)

acquisition of oocyte competence has been useful in explaining some empirical results as well as in devising new methods for refining current technologies. With the aim of increasing animal fecundity, the ultimate objective is to anticipate the time point at which an oocyte that is capable of being successfully fertilised *in vitro* can be isolated from the ovary. At present, successful development *in vitro* is limited to the use of fully grown oocytes from antral follicles; although, the vast majority of oocytes present in an ovary at any given time are enclosed in primordial and primary follicles. Current studies are preparing the way for the exploitation of this vast source of genetic material; however; considerable biological and technical hurdles still need to be overcome.

Such difficulties include the extended length of time required for a primary follicle to reach the antral stage, and the development of *in vitro* techniques that allow the appropriate diffusion of nutrients into ovarian fragments (67). Growth of the follicle to the antral stage ranges from a few days in the mouse to several weeks in domestic species, and this length of time makes it difficult to replicate follicle growth *in vitro* without degenerative processes taking place (55) (Fig. 4). However, the major obstacle is the limited knowledge of early follicular stage physiology. Live pups have been obtained from fresh (52) and frozen (44) mouse primordial follicles, but in cattle, the birth of live calves has been limited to oocytes isolated from early antral follicles (73). In the pig, a combination of xenotransplantation and *in vitro* culture was necessary for the fertilisation of pig oocytes isolated from primordial follicles (42). However, knowledge of early follicle and oocyte development is growing rapidly (27, 56) and is likely to result in a successful outcome in the near future.

The more distant future offers the exciting prospect of an unlimited source of oocytes from any given female obtained through the generation of oocytes from embryonic stem cells (ES). Embryonic stem cells are cell lines derived from preimplantation embryos. These cells have the remarkable ability to undergo continual self-renewal in long term culture, thus, preserving the ability of early embryos to differentiate into any given cell type. While the capacity of these cells to differentiate into most types of adult tissues is well established, recently it has been observed that these cells can form mature egg-like cells that are capable of developing into blastocysts (36). The recent findings of the existence of proliferative germ cells that sustain oocyte and follicle production in the postnatal mammalian ovary suggest a potential alternative source of unlimited oocytes (39). If confirmed, this would alter the current dogma that the oocyte population is finite. It may be possible to stimulate this germinal population and amplify the oocyte number available at birth. Both these scenarios are highly speculative, but imagination is the raw material that fuels scientific progress.

Mécanismes cellulaires et moléculaires régissant la qualité des ovocytes et impact sur l'efficacité de la reproduction des animaux d'élevage

F. Gandolfi, T.A.L. Brevini, F. Cillo & S. Antonini

Résumé
L'efficacité des systèmes d'élevage dépend de la fécondité des individus sélectionnés. Les techniques de reproduction repoussent constamment les limites physiologiques, mais si la fécondité des mâles est presque totalement exploitée, la physiologie reproductrice des femelles fait encore l'objet de recherches permanentes. Comme on ne peut pas agir sur le nombre de descendants auquel une femelle donnera naissance lors de chaque gestation, la méthode idéale consiste à prélever les descendants potentiels au début du développement et à les transférer à des receveuses de moindre valeur génétique. Plus le prélèvement a lieu précocement, plus important est le nombre de descendants que peut engendrer une femelle. Ainsi, le facteur limitant devient désormais le nombre d'ovocytes disponibles. Le présent article décrit comment les études détaillées sur la physiologie des ovocytes commencent à élucider l'enchaînement complexe par lequel un petit follicule primordial se transforme jusqu'à devenir un grand follicule ovulatoire contenant un ovocyte mûr. Progressivement, les limites de la manipulation des ovocytes ont été identifiées puis dépassées grâce aux traitements hormonaux adéquats administrés *in vivo* et à la supplémentation des milieux réalisée *in vitro*. Cela a conduit au développement de techniques de reproduction hautement efficaces et à la perspective d'avancées encore plus importantes dans l'avenir. Des découvertes surprenantes telles que le renouvellement folliculaire à partir de cellules souches ovariennes ou les lignées de cellules souches embryonnaires susceptibles de se différencier en ovocytes transforment rapidement nos prévisions.

Mots-clés
Acide ribonucléique messager maternel (ARNm maternel) – Adénosine monophosphate cyclique – Embryon – Fécondation in vitro – Fécondité – Follicule – Maturation – Ovaire – Ovocyte.

■

Los mecanismos celulares y moleculares que regulan la calidad del ovocito y su influencia en el rendimiento reproductivo del ganado

F. Gandolfi, T.A.L. Brevini, F. Cillo & S. Antonini

Resumen
La eficacia de los programas de reproducción está supeditada a que los ejemplares seleccionados presenten una elevada tasa de fertilidad. Las técnicas de reproducción fuerzan constantemente los límites fisiológicos, y aunque ya se ha extraído el máximo rendimiento posible del potencial reproductor de los machos, la fisiología reproductora de las hembras sigue siendo objeto de incansable investigación. Como es imposible modificar el

número de crías que una hembra puede llevar hasta el término de la gestación, el método idóneo consiste en retirar las futuras crías del útero al principio de su desarrollo y transferirlas a otras hembras que tengan menos valor genético. Una hembra podrá tener tantos más descendientes cuanto antes se realice la transferencia. En tal situación, el factor limitante será el número de ovocitos existentes. Los autores se refieren a una serie de detallados estudios sobre la fisiología del ovocito, gracias los cuales se empieza a desentrañar la compleja secuencia por la que un pequeño folículo primordial se transforma en un gran folículo ovulatorio que encierra un ovocito maduro. Progresivamente se han ido entendiendo y superando, gracias a tratamientos hormonales *in vivo* y a medios enriquecidos específicos *in vitro*, los límites de la manipulación de ovocitos. Ello ha dado lugar a técnicas reproductivas muy eficaces y a la perspectiva de avances aún mayores en el futuro. Nuestras expectativas evolucionan con rapidez a medida que se surgen descubrimientos como el de células madre ováricas capaces de reponer la población de folículos o el de linajes longevos de células madre embrionarias que pueden diferenciarse en ovocitos.

Palabras clave
Ácido ribonucleico mensajero materno – Embrión – Fecundación in vitro – Fertilidad – Folículo – Maduración – Monofosfato de adenosina cíclico – Ovario – Ovocito.

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