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Review
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Review

Successful in vitro maturation of oocytes: a matter of follicular differentiation

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Abstract

Folliculogenesis is remarkably similar in cattle and humans. In this review, we consider the known differences and provide a possible explanation for the greater success of oocyte in vitro maturation in cattle. Two different parallel processes that are critical for oocyte competence acquisition are examined. The first occurs in the follicle and in turn influences the oocyte, the second occurs within the oocyte itself and involves the gradual cessation of the transcription machinery with additional changes observable in the chromatin structure. We expect this insight to contribute to the improvement of human fertility programs based on in vitro fertilization, and particularly to the development of controlled ovarian stimulation protocols that yield more high-quality oocytes and thereby improve the clinical performance of treatments for infertility.

Summary Sentence

Successful in vitro maturation in cattle and humans starts with an optimally differentiated ovarian follicle.

Key words: human fertility, follicle-stimulating hormone, luteinizing hormone, follicular development, oocyte development, developmental competence, chromatin configuration, germinal vesicle.

Premise

The physiology of folliculogenesis in cattle and humans differs from that in mice in several ways [1, 2], the most obvious being the number of follicles ovulated per cycle, followed by the timing of the processes by which oocytes acquire all of the capabilities necessary to yield healthy offspring. These include resumption of meiosis, proper interaction with the sperm cell to produce a functional zygote, and timely activation of the embryonic genome, leading to successful implantation in the uterus and maintenance of gestation to term. These capabilities collectively are called oocyte quality or developmental competence. Though relying heavily on the competence of a single cell, folliculogenesis is the basis of reproduction throughout the animal kingdom. The focus of this review is the timing of follicular differentiation associated with the dominance period, which appears to be the principal determinant of the relationship between follicular events and oocyte quality [3].

Although the mouse is the most commonly used model of mammalian physiology and has been used widely in the field of reproductive biology, several authors suggest that mouse studies are not clinically relevant to human reproduction [2, 4–6]. In livestock as in humans, an oocyte has a very limited capacity to form an embryo until several events have occurred, none of which are fully understood and will be the main subject of this paper. Two different but parallel processes will be examined. The first of these occurs inside the follicle and influences the oocyte. The second occurs within the oocyte and involves the gradual shutdown of the transcription machinery and additional changes observable in the chromatin. These changes can be observed under the microscope, but also through molecular analysis of stored and translated RNA.

Acquisition of oocyte developmental competence

Oocyte maturation is a complex process involving much more than the nuclear maturation observed after the Luteinizing hormone (LH) surge or after the release of the full-sized gamete from its follicle. The nuclear changes that occur between the germinal vesicle (GV) stage and metaphase II represent only the final steps and the easiest to observe by microscopy. The complete process that leads to a competent oocyte begins a few days before ovulation and involves progressive changes in the follicular environment, including the antral stage, at the beginning of which the growing follicle reaches a diameter of about 2 mm [7, 8] and the oocyte acquires the ability to resume meiosis and become fertilizable. In this review, the competence of an oocyte means its ability to reach the blastocyst stage once fertilized. This is the most common definition used both in cattle breeding and in the study of human fertility.

The first point to emphasize is that although in vitro maturation (IVM) can take place spontaneously in fully grown oocytes released from antral follicles of any size, meiotic maturation occurs naturally only in large antral follicles (8.5 mm in cattle [9] and 11 mm in humans [7]) with functional LH receptors (on granulosa cells) following an LH surge. This difference is important, since completion of the oocyte differentiation program depends very much on the LH surge [3, 9]. Moreover, even if an oocyte matures and is fertilized in vivo, it still may fail to develop, especially following ovarian stimulation, as shown in cows in which the number of viable embryos recovered from the uterus is smaller than the number of ovulations, in spite of optimal insemination protocols [10].

It has been suggested that small follicles are lagging in development, while larger ones are too old at ovulation, both yielding lower quality oocytes. This hypothesis has gained support recently, since prolonging Follicle stimulating hormone (FSH) treatment has been shown to increase cattle embryo quality, while stopping it 84 h before ovulation leads to decreased quality [11]. In our research, we have determined an optimal FSH withdrawal period of about 48 h and have shown that shorter or longer durations both lead to decreased oocyte quality [12]. This variability is likely present in humans, in which oocytes aspirated 34 h after an induced LH surge or Human Chrorionic Gonadotropin (hCG) injection are not all competent as measured in terms of embryo formation and pregnancy following regular in vitro fertilization (IVF) [13].

In the bovine, initially oocytes were collected after in vivo maturation using laparoscopy [14] but with the development of ultrasound recovery, all laboratories and commercial units have moved to aspiration of immature oocytes followed by IVM and IVF [14]. After years of improvement, IVM now leads to impressive success rates exceeding 80% (based on blastocyst yield). However, these rates are achieved only if follicular differentiation has reached the optimal stage before harvesting the oocytes [12, 15], again suggesting that competence is achieved before the LH surge. With the new procedure, FSH is withdrawn (coasting) after several days of stimulation in a permissive basal LH environment with progesterone levels that prevent ovulation [12, 15]. If oocytes are aspired without this rise and fall in FSH level, blastocyst yield falls to about 30%, which can be obtained using slaughterhouse ovaries [16]. Similar treatment has proven successful in humans when FSH is replaced immediately by exogenous hCG injection [17]. In addition, in cases of ovarian hyperstimulation, complete cessation of FSH or any gonadotropin support for a few days often allows recovery of fully competent oocytes [18].

Chromatin configuration and oocyte developmental competence

The observations mentioned above indicate clearly that the oocyte acquires developmental competence just prior to ovulation. However, identifying oocytes that have achieved competence is extremely arduous. One indicator is large-scale chromatin remodeling, which occurs before meiotic resumption (while the oocyte is in meiotic arrest) and can be observed by microscopy in whole mount oocytes after DNA staining with fluorescent dyes [19, 20]. This has led to the identification of distinct stages in which the chromatin becomes more and more compacted and occupies a smaller area of the oocyte nucleus or GV. These configurations reflect oocyte competence status in all mammals studied so far [21].

In cows, four chromatin configurations corresponding to different stages of developmental competence have been described, namely GV0 to GV3. In the GV0 configuration, the chromatin appears mostly uncondensed and dispersed throughout the nucleoplasm. The appearance of few foci of condensation marks the transition to the GV1 configuration and further compaction into distinct aggregates characterizes the GV2 configuration. The highest level of compaction occurs in GV3, in which the chromatin appears as a single clump occupying a restricted area of the nucleus [22]. These stages accompany follicle development. Almost 90% of the oocytes isolated from early antral follicles (0.5–2 mm in diameter) have a GV0 configuration, while medium antral follicles (2–8 mm) contain virtually no GV0-stage oocytes but GV1, GV2, and GV3 stages in equal proportions. These are the follicles most commonly used for in vitro embryo production following IVM.

Various patterns of chromatin organization and compaction in several other mammals have been described. However, the nomenclature used to describe the changes in configuration is not uniform, due in part to species specificity. In mice, chromatin reconfigures from the nonsurrounded nucleolus (NSN) configuration to the surrounded nucleolus (SN) configuration, in which chromatin forms a rim around the nucleolus, which itself is inactive [23]. Configurations intermediate between these extremes include aggregates of condensed chromatin apposed to the nucleolus (partly NSN) and a partial perinucleolar chromatin ring (partly SN) [23, 24]. Human oocyte chromatin configuration also changes from dispersed to a rim condensed around the nucleolus [25], and one or two intermediate stages of aggregation around the nucleolar structure have been described [26, 27]. Murine, human, and bovine configurations are nevertheless similar, since the nucleolus of GV2 and GV3 oocytes is seen generally as an inactive remnant encapsulated by condensed chromatin [28]. The changes that are common to several species can be described as uncondensed (GV0), loosely condensed (GV1), intermediate (GV2), and condensed (GV3).

Chromatin configuration is not merely transient ultrastructure but a marker of gamete differentiation associated with various functional characteristics. In bovine oocytes, the transition from GV0 to GV3 corresponds to progressive transcription silencing [28], changes in epigenetic signatures such as overall methylation [29] and histone modification [30, 31] and changes in nuclear architecture and cytoplasmic organelle redistribution [28]. More importantly, the transition from dispersed to compacted chromatin is accompanied by progressive acquisition of meiotic and developmental competence [22, 28, 32]. Similar correlations have been described in mice and humans [24, 25, 27, 33, 34].

It is noteworthy that the changes in chromatin configuration also accompany significant changes in the transcriptome signature in the

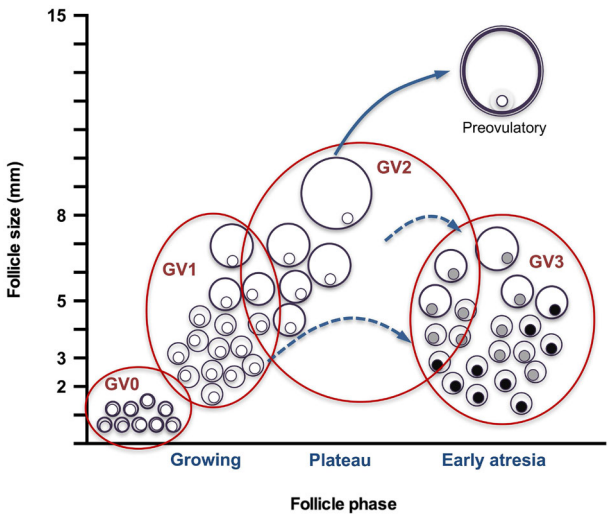

Figure 1. Schematic depiction of GV chromatin remodeling during bovine ovarian follicle development: Small spheroids colored white, light grey or black represent oocytes inside nonatretic, early atretic, or atretic follicles, respectively. Most growing phase follicles contain a GV1-stage oocyte, most plateau phase follicles contain a GV2-stage oocyte, and most early atretic phase follicles contain a GV3-stage oocyte. Modified from Dieci et al. [35].

oocyte and in the surrounding cumulus cells [30, 35]. For example, the abundance of transcripts from the H2A, H2B, H3, H4, and linker H1 family of histones in bovine GV oocytes clearly increases as chromatin compaction advances through the four stages [30]. This begins with an initial major drop in transcription during the GV0 to GV1 transition [28, 32]. In silico analysis has predicted interactions between specific histone transcripts and bovine stem-loop binding protein 2, which regulates mRNA translation throughout oogenesis, indicating active storage of selected histone-encoding transcripts, possibly to meet the requirements of the first three cell cycles following fertilization until activation of genes with embryological roles [30]. Meanwhile, the corresponding cumulus cell transcriptome also varies significantly [35], suggesting that chromatin configuration also reflects phases of follicle development (see Figure 1 and below). For example, ingenuity pathway analysis (in silico) has revealed a major change in transcripts involved in lipid metabolism during the GV0 to GV1 transition [35]. This is in agreement with recent findings that cumulus cell lipid content reflects oocyte quality [36, 37] both in cattle [38] and in humans [39]. It appears that oocyte survival is ensured by storing elevated levels of free fatty acids obtained from follicular fluid [40] to maintain the cumulus metabolic activities during meiotic progression [41]. Genes affecting cumulus "cell death and survival" functions also appear to be involved. Transcriptomic data analysis predicts inhibition of apoptosis in GV1/GV2-stage cumulus-oocyte complexes (COCs) and stimulation in GV3-stage COCs [35]. Caspase cascade analysis has shown that cumulus cells associated with GV0-stage oocytes are unlikely to undergo apoptosis, while those associated with oocytes at stages GV1, GV2, and GV3 are progressively more likely to do so [35].

Changes in oocyte chromatin configuration are apparently carried out through remodeling mechanisms, which regulate chromatin structure and accessibility. How these changes ultimately affect the above-mentioned cellular functions in the oocytes remains the subject of intensive study. We believe that optimizing techniques applicable to small samples such as oocytes and embryos will bring answers to this question in the near future.

Contribution of follicular cells to the process of chromatin remodeling

During folliculogenesis, oocyte and cumulus cells are involved in intense metabolic crosstalk arbitrated by paracrine factors and gap-junction-mediated communications. Recent bovine studies demonstrate that large-scale changes in chromatin configuration are related to gap-junction functional status through a cyclic adenosine monophosphate (cAMP) dependent mechanisms [32, 42, 43]. In COCs isolated from early antral follicles, the maintenance of functional gap-junction communications promotes oocyte growth, gradual transcriptional silencing, large-scale chromatin remodeling and competence acquisition, all of which are controlled via oocyte cAMP [32].

Several studies have been focused on mimicking the contribution of follicular cAMP and the synchrony between nuclear and cytoplasmic maturation in attempts to perfect in vitro oocyte culture. This derives from the concept of "prematuration" that is the process occurring from the time a follicle is selected to become dominant and are completed shortly before the LH surge initiates final maturation [44]. In the prematuration design, oocytes are held in prophase I (GV stage) using different molecules that act on the intracellular level of cyclic nucleotides associated with meiosis resumption in order to promote the acquisition of complete oocyte developmental competence prior to IVM [45]. A similar perspective characterizes studies conducted on human oocytes [27, 46, 47]. A series of bovine studies demonstrates that prematuration in the presence of cilostamide, 3-isobutyl-1-methyl-xanthine (phosphodiesterase inhibitors), or natriuretic peptide precursor C (which stimulates cyclic guanosine monophosphate (cGMP) synthesis by binding its cognate receptor natriuretic peptide receptor 2, NPR2) plus FSH at physiological concentrations stimulates the opening of gap junctions and gradual chromatin transition. This transition ultimately increases oocyte embryonic developmental competence after standard IVM and IVF, as evaluated in terms of blastocyst quality parameters such as cell number and hatching rate [35, 42, 43, 48, 49]. However, as several groups have demonstrated over the past decade, the resulting increase in embryo quality, though statistically significant, is modest. This has led some to suggest that the heterogeneity of the ovarian follicle population in naturally cycling animals affects the success obtained with the different bovine embryo production systems [50]. A study conducted using an oocyte selection enriched on the basis of chromatin configuration has shown that prematuration may be beneficial for the developmental competence of GV1 oocytes but detrimental for that of GV3 oocytes [35].

The involvement of the follicle in the acquisition of developmental competence is illustrated dramatically by studies using bovine oocytes obtained from slaughterhouses. These oocytes are recovered from ovaries of unstimulated animals at random stages of the estrous cycle. The bovine estrous cycle consists of a 15-day luteal phase and a 6–7-day follicular phase. Throughout the luteal phase, two or three follicular waves are generated, each characterized by the appearance of a dominant follicle and the regression of subordinate follicles, since cattle are mono-ovulatory, as are humans [51]. Ovaries obtained from slaughtered cows thus contain all types and sizes of antral follicles representing all stages of oocyte development. For example, oocytes from 2–8-mm follicles are heterogeneous in GV chromatin distribution as well as competence [22, 35]. It is interesting that oocytes in the GV3 configuration show initial degradation of the gap junction coupling between the follicle and oocyte compartments as well as early signs of atresia (based on ultrastructure),

Table 1. Apparent association of bovine follicle size category and growth status at OPU with oocyte developmental potential.[†]

	Follicle status (%)				Blastocyst yield (estimated %)			
Size cat.	Growing[1]	Plateau[2]	Atresia[3]	Ref.	Growing	Plateau	Atresia	Ref.
<3 mm	85	10	5	[22, 35]	Nil	<25	Low[4]	[77]
3–5 mm	30	40	30	[22, 35]	Nil	Low	Low	[77]
5–9 mm	10	30	60	[22, 35]	Nil	25–40	<25	[56]
9 mm to ovulation	NA	NA	NA	NA	Low	25–40	<25	[56]
FSH withdrawn after 3 days	10	80	10	(*)	Low	>80	Low	[12, 15]

[†]Based on published data [22, 35] and our unpublished results (*).
[1]mGV chromatin was at stage 1 in all categories.
[2]GV chromatin was at stage 2 in all categories.
[3]GV chromatin was at stage 3 in all categories.
[4]Low means close to nil.

although they still have a relatively high developmental capability [22, 28]. This is in agreement with previous studies in which a correlation was identified between COC morphology and developmental competence [52, 53]. In particular, oocytes within COCs with compact cumulus and homogeneous ooplasm are less competent than those in which the ooplasm appears granulated and the outer layers of cumulus cells exhibit the slight expansion often seen in early atretic follicles. This suggests that oocytes inside follicles in the early stages of atresia are more competent than those inside growing follicles [52]. The possible association of developmental competence with follicle differentiation has been explored in conjunction with FSH withdrawal [12, 15]. In compact COCs, the oocyte tends to be in the GV1 chromatin configuration (loosely condensed), while in COCs with slight expansion and/or granulated cytoplasm the oocyte chromatin is in either the GV2 or the GV3 configuration only [35]. It is interesting that in the case of GV1-stage oocytes, standard IVM without 6 h of prematuration leads to poor preimplantation development and yet the same pretreatment decreases the competence of oocytes that have reached the GV2 or GV3 stage [35].

These findings suggest strongly that in naturally cycling animals, follicle size is not a reliable criterion for selecting a population of oocytes that is homogeneous in terms of chromatin configuration. The GV1 to GV2 transition, which marks the acquisition of high embryonic developmental potential, occurs in oocytes almost regardless of the antral size reached before FSH levels decline and the dominant follicle emerges [35]. Once the FSH level peaks within a follicular wave and then begins to decline, the atretic events start and the GV2 to GV3 transition eventually occurs. The timing of such a sequence would result in oocyte chromatin compaction regardless of whether the follicle ovulates or undergoes atresia. This supports a previously proposed hypothesis that chromatin condensation is complete in oocytes that became fully competent during follicular growth and were collected from follicles undergoing the early events of atresia [22, 28], the latter being corroborated by gene expression in cumulus cells [35]. This is summarized in Figure 1 and is consistent with the finding that 33% of oocytes obtained from follicles in the early stages of atresia have a relatively high developmental potential [12, 15, 52]. If such oocytes were at the GV2 stage and therefore maximally competent, this would explain the extraordinarily stable blastocyst yield obtained by IVM since 1995. Oocytes in growing follicles would be mainly at the GV1 stage, while those in plateau phase follicles (low FSH) would be at GV2 and those in early atretic follicles at GV3, with respectively low, high, and medium developmental competence.

The interesting paradox described above is that neither the size nor the healthiness of the follicle is the sole determinant of oocyte quality or competence. Indeed, both small follicles (2–3 mm) and early atretic follicles may yield embryos and newborns [52, 54, 55]. Furthermore, embryos with increased competence can be obtained from oocytes harvested from larger follicles (>9 mm in cattle), especially those expressing LH receptors [56], the latter indicating that the gradual progression toward developmental competence is complete. Analysis of follicle size, stimulation conditions, and time spent in the dominant phase (LH receptor) indicates that several factors contribute to the progression towards a flawless oocyte with full developmental competence. However, the complexity of the process is greater than meets the eye, since oocyte competence can be reduced or lost suddenly if the follicle does not proceed to timely ovulation [11, 12, 56–60]. It is now recognized that at any follicle size above 3 mm, three phases of follicular differentiation occur, namely growth, plateau, and atresia, and these phases correspond to the rise and decline of oocyte quality [61, 62]. A speculative association between chromatin configuration and follicle size is presented with estimated blastocyst yield in Table 1.

Bovine to human comparisons

In the field of human fertility, IVM needs semantic clarification [63]. Maturation of human oocytes aspirated without prior ovarian treatment was attempted a few times [64, 65], while most investigators worked with GV-stage oocytes collected following regular controlled ovarian stimulation (COS) cycles [66, 67]. Meanwhile, a McGill University group working with nonstimulated patients examined the effect of 34 h of IVM following injection of hCG and aspirating from the first follicle to reach 11–12 mm [68]. These three types of IVM protocols produce oocytes with quite different characteristics. The first of these is analogous to the ovum pickup (OPU) procedure developed for slaughterhouse material or nonstimulated, nonsynchronized animals. The follicles aspirated are nondominant, early or late atretic, and the granulosa cells are not yet expressing LH receptors. Each year, hundreds of thousands of oocytes are recovered from cows without ovarian stimulation or synchronization, notably in Brazil where this is the main system of reproduction on many large commercial farms [69]. Such oocytes have been used in research for at least three decades [55] and their quality is well known, the yield of transferrable embryos being 20–40% and 40–50% of transfers leading to pregnancy. It is also known that using ultrasound transvaginal aspiration, mostly early and late atretic oocytes are recovered, the former yielding more embryos than the latter, in which the cumulus cell investment is often partially lost [52]. There is reason to believe that the situation would be similar in humans, although the number of studies is limited [70, 71]. In the second type

Table 2. Apparent association of human follicle size category and surmised GV stage with oocyte developmental potential.

Follicle size (mm)	Matured (% of total)	Blastocyst yield (%)	Reference	Surmised stage
2–5	56	35	[78]	GV-1-2-3
2–6		30	[75]	GV-1-2-3
6–9 (subordinate)	75	12	[66,73]	GV-2-3
>9–12		23	[79]	GV-1-2-3
9–12 + hCG		40	[79]	M-II
>9 (GV, COS)	52	5	[67]	GV1
2–10	48	27	[80]	GV-1-2-3

of protocol, IVM is performed on oocytes collected in the context of a regular COS. Isolated typically from follicles that did not respond to the hCG injection, these oocytes tend to be surrounded by nonexpanded cumulus cells and/or are still at the GV stage. Several groups have tried to rescue them with a further 24, 36, or 44 h of IVM in a culture system similar to that used for cattle oocytes. Their success has been very limited [66] and currently very few clinics if any still try to produce embryos in this manner. Follicles containing such oocytes are most likely in the rapid growth phase, stimulated by several days of FSH treatment but not differentiated enough to express functional LH receptors in numbers above the threshold of response to the ovulation trigger. Bovine oocytes aspirated from follicles in this phase are less competent than those harvested later on, even from early atretic follicles of the same size [52, 57], and oocytes in growing follicles may be still at the GV1 stage (Table 2). It should be noted that oocytes with the GV still intact after exposure to hCG are often used in research protocols designed to optimize IVM. This has contributed to confounding the effects seen in comparative studies conducted on IVM/IVP with oocytes isolated from normal cycling animal models and has been discussed in recent debate on defining clearly what should not be considered as IVM [63, 72]. A third type of protocol although not sustained by statistical analysis further confuses the issue. In this procedure, hCG is injected with minimal or no FSH pretreatment and aspiration is performed 30–34 h later [68]. The resulting dominant follicle is often large enough (11–12 mm) to have sufficient LH receptors to start the process of ovulation and accordingly a mature oocyte with an expanded cumulus is recovered. Subsequent IVF is performed twice: immediately with the mature oocyte and 2 days later with immature oocytes kept in culture. Based on our experience with livestock, the mature oocyte is more likely to become an embryo and lead to pregnancy, especially when embryos of different stages are transferred to the same uterus [71].

Subordinate follicles that were still growing at the time of the hCG trigger cannot respond to LH since they received less FSH and have begun the atretic process, which is not immediately detrimental if our experience with cattle is any indication. In fact, it is likely that the LH (hCG) trigger itself promotes atresia by stimulating androgen production in theca cells of follicles in which the granulosa cells bear no LH receptors [3]. If the dominant follicle is already functional (12 mm or larger) the subordinates have already started the atresia process and will be on hold for the 30–34-h period prior to aspiration. These oocytes are associated with cumulus cells exhibiting various degrees of dispersion or apoptosis, suggesting that hCG triggering did not have the same effect as it did on the dominant follicle [71]. The procedure has been tried in many laboratories around the world but is now seldom used for regular patients since the results are not superior to regular IVF and smaller numbers of eggs are produced [70].

Recent work [73] has demonstrated convincingly a crucial upper limit on dominant follicle diameter. The observations of Son's group [73] make it clear that human COCs collected from 6–12-mm follicles when the dominant follicle was larger than 12 mm had very little developmental competence.

A modified IVM procedure has been developed to include a few days of FSH support to increase follicle recruitment before aspiration [74]. When OPU is performed before FSH coasting (withdrawal), the recovery rate is poor because COCs remain attached solidly to their follicles (De Vos, personal communication). The oocytes that are recovered under these conditions may have a less compacted cumulus but are comparable in developmental capacity to what is observed in cattle treated similarly [57]. Again, these oocytes are harvested while the follicular process is incomplete and are therefore less competent.

The quality of oocytes obtained using the three IVM protocols described above cannot be stated with certainty, due to the limited reproductive data available. Table 2 shows blastocyst yields ranging from 5% to 30% based on the number of fertilized or cleaved embryos, which is somewhat lower for mice or livestock. The best results for true IVM come surprisingly from ovariectomy patients who received no treatment before oocyte collection, but no embryo was transferred in these cases [75]. The poorest results come from GV oocytes recovered during a stimulated cycle 34–36 h after hCG, probably indicating that such follicles were recruited later, were still growing or bore insufficient LH receptor (Table 2). We speculate that ovaries selected at random would have a balanced distribution of growing (mainly GV1), plateau (mainly GV2), and early atretic (mainly GV3) follicles (Figure 1). With such a distribution, we would obtain the same blastocyst yield (30%) as from cows selected randomly or from slaughterhouse ovaries. If oocytes were collected from patients while a follicle was establishing dominance (>9 mm), this follicle would be at the GV1 or GV2 stage, depending on how many days into the process before ovulation. Oocytes from subordinate follicles would be at the GV2 stage for 1–2 days and then would enter final atresia and the GV3 stage. Immature oocytes obtained 34–36 h post-hCG in the context of in a stimulated cycle would most likely be in the GV1 stage, due to the follicle not responding adequately to the ovulation trigger. This is the worst-case scenario if these follicles are otherwise totally healthy and responsive to stimulation by FSH. Under such conditions in the cow, the larger growing follicles contain oocytes of the lowest quality [57]. What has not been done is the collection of immature oocytes from large (with LH receptors) growing follicles before the LH surge. There is no reason to expect these follicles to respond to hCG and generate mature eggs in vivo within 34 h. Based on animal models, we would even predict that without proper coasting (FSH withdrawal), these oocytes would be less competent after IVM than those matured in vivo.

Conclusions

Bovine oocytes perform better in IVM than human oocytes do, mainly because the oocyte harvesting is made from follicles that have acquired LH receptors (dominant), which can optimize differentiation in absence of FSH. In human fertility treatment, such follicles mature in vivo without FSH arrest. When FSH is replaced by low doses of LH (hCG), human oocytes in IVM appear to be as competent as bovine oocytes [76]. We believe that if the concepts outlined in this review were applied to human IVF programs, COS protocols could be adjusted to make it easier to obtain high-quality oocytes, especially for patients who do not respond well to standard

COS protocols or present a risk of developing the hyperstimulation syndrome.

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