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# Potential genetic improvement of cattle by fertilization of fetal oocytes *in vitro*

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## Introduction

"A hen is only an egg's way of making another egg" (Butler, 1877) but turning life upside down in 'chicken and egg' debates has not often been extended to mammals. Indeed, most of us at this meeting have become interested in the gametes of farm animals and the process of fertilization largely because of our interest in the embryos and animals to which they give rise. This is a logical and practical reason for the interest, brought about by the rapid advances in the various subjects under discussion at this symposium. However, in a broader biological perspective, conferences like this do represent a remarkable shift of emphasis of study from the diploid to the haploid generation, as well as to the mechanisms controlling the transitions between the generations (Fig. 1).

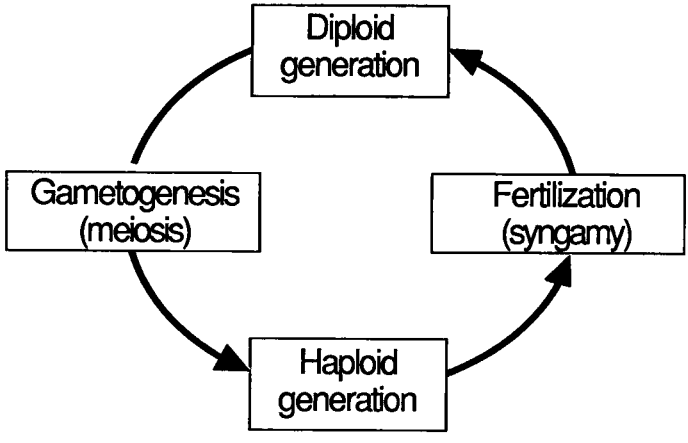


**Fig. 1.** The alternation of generations.

The interval between generations is one of the key elements in any approach to the genetic improvement of livestock. Bringing the egg to the forefront of a consideration of the alternation of generations may also help define other ways in which manipulation of the egg might be used as a tool for genetic improvement.

Reduction of the generation interval is one of the means by which multiple ovulation and embryo transfer is already being used for genetic improvement of cattle (Nicholas & Smith, 1983). However, this approach depends on the use of cows or heifers that are old enough to ovulate secondary oocytes from mature ovarian follicles. The fact that primary oocytes from much smaller antral follicles can now be successfully matured and fertilized *in vitro* suggests that oocytes from

primordial follicles, oogonia, or even primordial germ cells might become amenable to similar procedures. Some of the advantages that growing and maturing oocytes *in vitro* could bring to livestock production have been discussed by Eppig & Schroeder (1986). However, since the number of primary oocytes reaches a peak prenatally, the potential of using in-vitro fertilization of fetal oocytes in cattle breeding schemes bears consideration.

In this paper the genetic advantages and biological difficulties of using fetal bovine oocytes will be discussed against a brief background description of relevant aspects of oogenesis, folliculogenesis and current methods of maturing and fertilizing oocytes *in vitro*.

## The genetic advantages

Cattle breeders have long wished to produce 'replicas' of their best, selected, progeny tested sires and some possible approaches to achieving this goal have been discussed by Markert & Seidel (1981). Van Raden & Freeman (1985) evaluated the rates of genetic improvement that could result from sire × sire 'matings' and showed that they would result in substantial gains in comparison with conventional breeding systems. However, it has now been shown that proposals to produce progeny from crossing two sires by replacing a female pronucleus with a second male pronucleus will not be realizable. This is because the phenomenon of parental imprinting dictates that both male and female pronuclei be present if a zygote is to develop to term (Surani *et al.*, 1987).

An alternative means of combining the genetic merits of selected bulls relatively rapidly could be a backcrossing system using fetal oocytes fertilized *in vitro* to provide embryos for transfer to recipients whose genetic quality would be irrelevant (Fig. 2). It should be noted that backcrossing to the same sire would not be desirable because it would lead to the deleterious effects of inbreeding at high levels (coefficients of inbreeding of 0, 0·25 and 0·375 for the 3 generations shown in Fig. 2). The average genetic merit of the offspring born from the fetal oocytes would rapidly become equal to the average merit of the selected sires; those offspring above and below average would be identified by a further round of progeny testing and so the genetic improvement would be continuous. The interval between successive backcrosses in this scheme would depend on how early in fetal or post-natal development the bovine oocyte could be fertilized *in vitro*. For the purposes of this discussion, this has been projected to be possible at 90 days gestation.

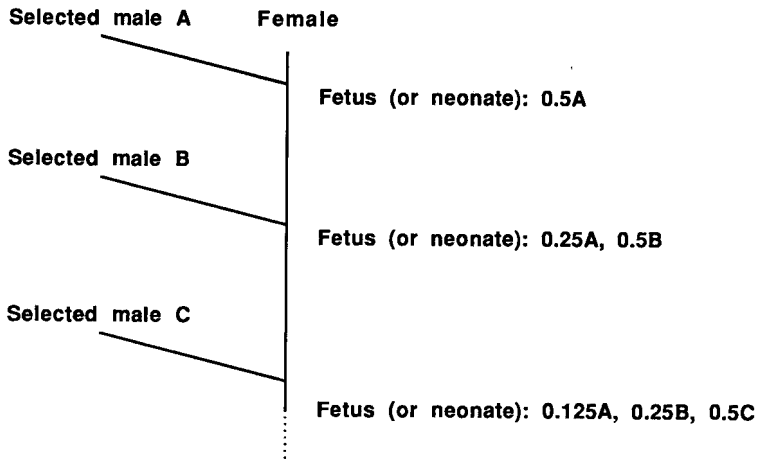


**Fig. 2.** An hypothetical backcrossing system to selected males using fertilization of fetal oocytes *in vitro*.

Table 1 summarizes calculations of the genetic change that could be achieved in milk yield in dairy cattle by using in-vitro fertilization of fetal oocytes by selected, progeny-tested sires. For comparison, Table 1 also shows calculations, made on the same basis, of the changes that can be achieved through conventional progeny testing (Nicholas & Smith, 1983), efficient progeny testing (Woolliams & Smith, 1988), and by multiple ovulation of adult and juvenile animals and embryo transfer (Nicholas & Smith, 1983). It can be seen that the potential benefits of the in-vitro fertilization scheme should be slightly better than those of even efficient progeny testing schemes or multiple ovulation of adults and embryo transfer, but not as great as those obtainable through multiple ovulation of juveniles and embryo transfer. One advantage of in-vitro fertilization that is not shown in Table 1 is that the unreliable dam-to-sire path (e.g. due to preferential treatment of bull-dams) would be avoided. Van Raden & Freeman (1985) calculated that sire × sire mating would give 1·1 to 1·3 times the rate of genetic gain that could be obtained through an efficient progeny-testing system, allowing for the reduction in genetic variance due to the selection of sires.

**Table 1.** Annual rate of genetic change (standard deviation units) theoretically possible in milk yield by different breeding systems

| Breeding system | | Path: Sire–sire | Sire–dam | Dam–sire | Dam–dam | Annual response* |
|---|---|---|---|---|---|---|
| Conventional progeny test† | | | | | | |
| Age (years) | (L) | 6·5 | 7·5 | 6 | 5 | |
| Accuracy | (r) | 0·88 | 0·8 | 0·65 | 0·6 | |
| Proportion selected | (p) | 0·1 | 0·2 | 0·10 | 0·9 | |
| Selection intensity | (i) | 1·76 | 1·4 | 1·76 | 0·2 | 0·081 |
| Efficient progeny test | | | | | | |
| Age (years) | | 6·5 | 6·5 | 6 | 5 | |
| Accuracy | | 0·88 | 0·8 | 0·65 | 0·6 | |
| Proportion selected | | 0·04 | 0·04 | 0·01 | 0·9 | |
| Selection intensity | | 2·15 | 2·15 | 2·66 | 0·2 | 0·117 |
| Multiple ovulation of adults and embryo transfer | | | | | | |
| Age (years) | | 3·7 | 3·7 | 3·7 | 3·7 | |
| Accuracy | | 0·55 | 0·55 | 0·65 | 0·65 | |
| Proportion selected | | 0·13 | 0·13 | 0·25 | 0·25 | |
| Selection intensity | | 1·65 | 1·65 | 1·27 | 1·27 | 0·117 |
| Multiple ovulation of juveniles and embryo transfer | | | | | | |
| Age (years) | | 1·8 | 1·8 | 1·8 | 1·8 | |
| Accuracy | | 0·43 | 0·43 | 0·43 | 0·43 | |
| Proportion selected | | 0·13 | 0·13 | 0·25 | 0·25 | |
| Selection intensity | | 1·65 | 1·65 | 1·27 | 1·27 | 0·174 |
| In-vitro fertilization of Day-90 fetal oocytes by selected progeny tested sires | | | | | | |
| Age (years) | | 6·5 | | | | |
| Accuracy | | 0·88 | | | | |
| Proportion selected | | 0·04 | | | | |
| Selection intensity | | 2·15 (0·875)‡ | | | | 0·127 |

*Annual response $= \frac{\sum irh}{\sum L} \cdot \sigma$, where heritability ($h^2$) = 0·25.

†Progeny test on 50 daughters.

‡0·875 = (0·5 + 0·25 + 0·125) as in Fig. 2.

Table 2 summarizes analogous calculations and comparisons for the efficiency of lean growth in beef cattle. As can be seen, performance and progeny testing schemes produce equivalent responses which can be doubled with multiple ovulation and embryo transfer (Land & Hill, 1975). The in-vitro fertilization schemes should produce high responses, but less than in schemes with multiple ovulation and embryos transfer.

**Table 2.** Annual rates of genetic change (standard deviation units) theoretically possible for efficiency of lean growth in beef cattle by different breeding systems

| Breeding system | | Sire | Dam | Annual response† |
|---|---|---|---|---|
| Performance test | | | | |
| Age (years) | (L) | 2 | 4 | |
| Accuracy | (r) | 0·55 | 0·55 | |
| Proportion selected | (p) | 0·1 | 0·5 | |
| Selection intensity | (i) | 1·76 | 0·8 | 0·128 |
| Progeny test‡ | | | | |
| Age (years) | | 4 | 4 | |
| Accuracy | | 0·82 | 0·55 | |
| Proportion selected | | 0·1 | 0·5 | |
| Selection intensity | | 1·76 | 0·8 | 0·129 |
| Performance test with multiple ovulation and embryo transfer* | | | | |
| Age (years) | | 2 | 2 | |
| Accuracy | | 0·6 | 0·6 | |
| Proportion selected | | 0·1 | 0·25 | |
| Selection intensity | | 1·76 | 1·27 | 0·249 |
| In-vitro fertilization of Day-90 fetal oocytes using progeny-tested sires | | | | |
| Age (years) | | 4·25 | — | |
| Accuracy | | 0·82 | — | |
| Proportion selected | | 0·1 | — | |
| Selection intensity | | 1·76 (0·875) | — | 0·163 |
| In-vitro fertilization of Day-90 fetal oocytes using preformance-tested sires | | | | |
| Age (years) | | 2·25 | — | |
| Accuracy | | 0·55 | — | |
| Proportion selected | | 0·1 | — | |
| Selection intensity | | 1·76 (0·875) | — | 0·205 |

*16 Embryos/donor.

†Annual response $= \frac{\sum irh}{\sum L} \cdot \sigma$, where heritability ($h^2$) = 0·3.

‡Progeny test on 25 daughters.

These calculations, of course, depend on assumptions of specific details and should be viewed as being indicative rather than exact for the various systems. Reduced variances due to selection result in lower responses but, since this applies to all the schemes, the relative values should be appropriate. These provisos notwithstanding, the potential benefits of an ability to use in-vitro fertilization of fetal oocytes are evidently considerable. The scheme would avoid the unreliability of female selection in the field; for beef, it offers a means of breeding high-merit young sires for natural service in commercial herds. Also for beef, it would obviate the testing of females, making more testing facilities available for males, thus allowing more intense selection and still more genetic change. For both dairy and beef cattle, the scheme would produce high-merit embryos for commercial use, thereby reducing the genetic lag in dissemination of the benefits.

Since these benefits seem worth pursuing, the biological obstacles that will need to be overcome should be considered.

## The biological difficulties

For oocytes to be fertilized *in vitro*, the more remote they are from the time of natural ovulation at the time of collection, the more complex are the events that need to be accomplished during culture. Any use of fetal oocytes will need to take this into account and the developmental complexities that will need to be negotiated *in vitro* are underlined by considering how secondary oocytes are derived from primordial germ cells *in vivo* through the processes of oogenesis and folliculogenesis. These events have been previously reviewed in relation to the superovulation of cattle (Baker & Hunter, 1978). However, since solution of the problems of obtaining viable oocytes from antral follicles in adult cattle is progressing so well, discussion of the possibility of using fetal oocytes can be concentrated on the earlier stages of oogenesis and folliculogenesis, stages which have been excellently described for cattle and sheep by Rüsse (1983).

### *Oogenesis*

Primordial germ cells (or gonocytes) divide mitotically, differentiating into oogonia in females, spermatogonia in males. In generating oocytes, oogonia undergo the two cell divisions of meiosis in order to reduce their diploid chromosome and DNA complement (2n) to the haploid state (n) and to provide an opportunity for resegregation of the genetic information in that material. Once an oogonium enters prophase of the first meiotic division, it becomes known as a primary oocyte; after completion of the first meiotic division and entry into the second, it is a secondary oocyte. After the two meiotic divisions, the resulting haploid cells should strictly be known as ootids (by analogy with spermatids), but the term is rarely used.

The essential difference between mitosis and meiosis lies in the prolonged and specialized first meiotic prophase. Throughout most of this prophase, the primary oocytes possess a 4n complement of DNA because they underwent DNA synthesis either during the S phase or during the course of early prophase. This tetraploidy is one of the factors exploited in new methods of separating germ cell populations by flow cytometry (Larsen *et al.*, 1986). This could be of practical importance if fetal oocytes are to be used. Completion of the first meiotic division separates bivalent whole chromosomes into the daughter cells (secondary oocytes and first polar bodies) which, therefore, each contain n chromosomes and 2n DNA.

The second meiotic division involves an extremely brief prophase (or perhaps none at all in oogenesis) and separates the constituent chromatids of each haploid set of bivalent chromosomes. Every germ cell entering meiosis therefore has the potential to produce 4 dissimilar haploid cells; 4 spermatids or an ovum (ootid) with 2, or occasionally 3, polar bodies.

Rüsse (1983) has separated the process of oogenesis into six periods: (1) the primordial germ cell period; (2) the oogonial period; (3) meiotic prophase I; (4) the isolation of the oocyte from the germ cell cords; (5) follicle activation and differentiation of the ovum; and (6) the tertiary follicle and completion of meiosis (Table 3).

In male and female farm animals, the primordial germ cells are presumed to first become recognizable in the dorsal endoderm of the yolk sac, near the point of development of the allantois, as in man (see Byskov, 1982) but it has not yet been possible to say whether they originate from ectoderm, mesoderm or endoderm (Eddy *et al.*, 1981). They migrate to, and into, the genital ridge over the mesonephros before sexual differentiation of the gonads has occurred (by about 40 days gestation in the cow).

The 'explosive' proliferation of female germ cells early in fetal development is illustrated for the cow in Fig. 3. At first, this involves mitotic division of the primordial germ cells and separation of

**Table 3.** Events that could affect the use of germ cells from fetal bovine ovaries for fertilization *in vitro* (data from Rüsse, 1983)

| Event | Stage of fetal development | |
|---|---|---|
| | Crown–rump length (cm) | Gestation day |
| Formation of genital ridge | 1·0 | 30 |
| Sexual differentiation of gonad | 2·5 | 40 |
| Development of oogonial clusters | 5·5 | 57 |
| Synchronization of mitosis in: | | |
| 4 oogonia | 7·0 | 62 |
| 8 oogonia | 9·8 | 74 |
| 16 oogonia | 12·5 | 82 |
| Meiosis starts | 12·5 | 82 |
| Follicle isolation starts | 16·0 | 90 |
| Activation of primordial (primary) follicles | 32·5 | 140 |
| Secondary follicles appear | 65·0 | 210 |
| Tertiary follicles appear | 74·0 | 230 |
| Birth | ~100·0 | 280 |

identical daughter cells. Subsequently, the final division of each primordial germ cell gives dissimilar daughter cells. One immediately undergoes mitosis and produces an oogonial cell line; the other remains in interphase and may be a 'stem cell' capable of continuing mitosis later to produce clusters of oogonia dividing synchronously (Rüsse, 1983). The fetal ovary therefore contains germ cells in various developmental states during their rapid proliferation, as well as somatic cells.

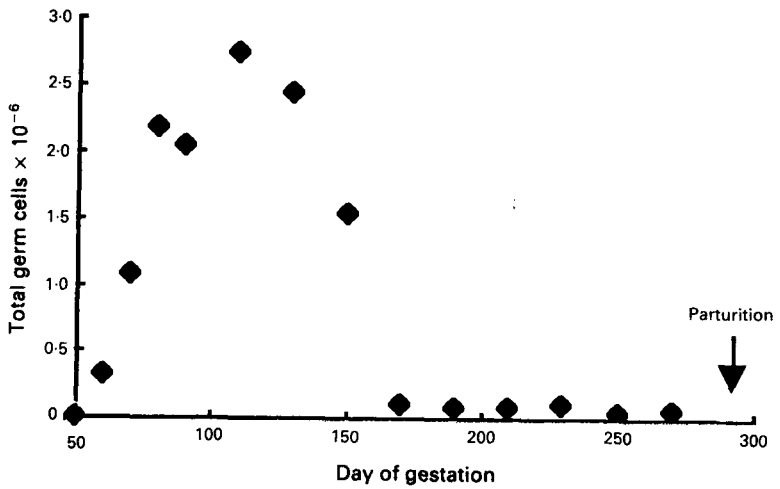


**Fig. 3.** Total numbers of germ cells in the prenatal bovine ovary at various stages of gestation. Data from Erickson (1966a).

Synchronized mitotic cell division has been observed in clusters of up to 16 oogonia (Rüsse, 1983), implying that only about 4 such divisions are possible before the oogonia enter meiosis. In the cow, meiosis begins to occur from about 72–82 days gestation, during the steep ascent of the

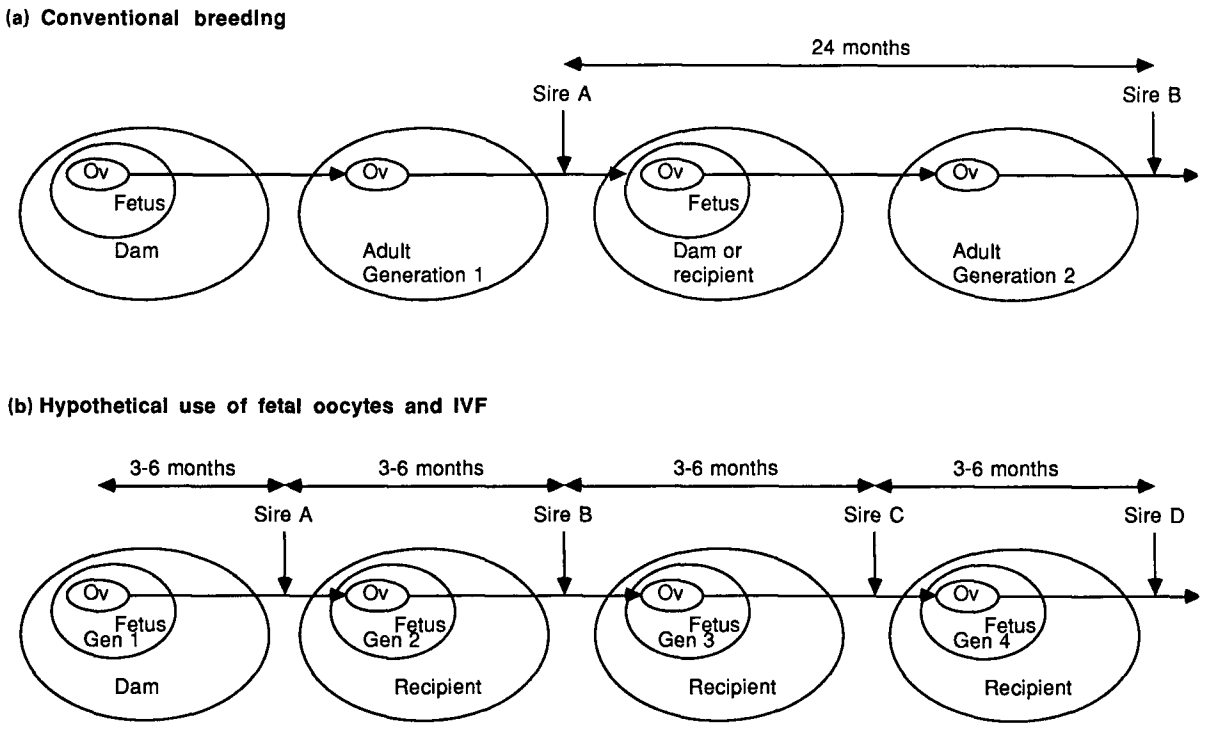


**Fig. 4.** Comparison of animal breeding strategies using (a) conventional methods and (b) in-vitro fertilization of fetal oocytes.

population curve shown in Fig. 3. The end of mitotic multiplication means that the total number of oocytes that the female will ever have becomes fixed once and for all during this period of oogenesis between about 80 and 170 days gestation. In histological sections, primary oocytes are readily distinguishable from oogonia (Rüsse, 1983).

Two important delays make the rate of meiosis during oogenesis very much slower than during spermatogenesis. The first delay occurs before diakinesis during prophase of the first division and extends from the time the primary oocyte enters meiosis in fetal life to the time when, normally, it is induced to resume the division immediately before ovulation. Consequently, the DNA in the oocytes ovulated by an adult cow would have been synthesized months or years before (during the S phase preceding meiosis) and open to possible deleterious modification throughout that period. The leptotene, zygotene, pachytene and diplotene stages of meiotic prophase are descriptive of the appearance of chromosomes during recombination and crossing over. However, the successive stages also demarcate other cellular changes, including the loss of intercellular bridges between the oocytes as they gradually become isolated in association with follicular cells of somatic origin (Rüsse, 1983; Table 3).

Once the primary oocyte and its follicular cells are isolated, the oocyte is said to enter a resting phase. During this phase, the chromosomes in the oocyte unwind and the cytoplasmic organelles in both the oocyte and follicular cells become reduced in number. The resting phase persists until follicular cells become activated. With activation, growth and differentiation of the primary oocyte is resumed. This occurs in a proportion of primordial follicles after 140 days gestation as described below, under 'Folliculogenesis' (Table 3).

During the early stages of growth of the primary oocyte, there is synthesis of the RNA which probably acts as a messenger for protein synthesis in the oocyte itself and during the early cleavage stages after fertilization. The cytoplasm of the growing oocyte contains all the required organelles for protein and mucopolysaccharide synthesis (Baker, 1982). In cattle, the high rate of RNA synthesis that characterizes oocytes isolated from early antral follicles, as well as later RNA synthesis in the oocytes from larger follicles, are considered necessary for the storage of information essential to the restarting and completion of meiosis (Motlík *et al.*, 1984; Motlík & Fulka, 1986). Once the oocyte reaches its maximum size, its nucleolus undergoes changes that are recognized morphologically and rRNA synthesis decreases substantially (Crozet *et al.*, 1986). The rRNA produced by the growing oocyte is of an unusually stable form; in the pig, for example, it must serve for protein synthesis by fully grown oocytes over a 15–20-day period between their attainment of full size (in 2–3 mm follicles, say 2–4 days after preceding ovulation) and the time of activation of the embryonic genome after fertilization (Motlík & Fulka, 1986). It is therefore misleading to regard the growing, dictyate primary oocyte as 'resting' metabolically; indeed, from morphological, physiological, and metabolic standpoints it can be compared with a somatic cell in the G1 phase of the cell cycle (Motlík & Fulka, 1986) and so its culture requirements might also be similar.

When the resumption of meiosis is stimulated, the primary oocyte passes from the dictyate, or the 'germinal vesicle' stage, into diakinesis. Following germinal vesicle breakdown, the chromosomes become orientated in the metaphase I configuration, anaphase and telophase rapidly follow, extruding the first polar body (PB-1) and thereby forming the secondary oocyte. In contrast to the extremely long prophase of the first meiotic division, there is virtually no prophase to the second, so metaphase II follows directly, It is at this point that the second interruption of meiosis occurs, until resumption following activation of the egg by the fertilizing spermatozoon.

The decline in female germ cell numbers from the peaks typified in Fig. 3 involves oocyte atresia in differing circumstances (see Prépin *et al.*, 1985). During the period of oogenesis, massive losses are incurred amongst oogonia in mitotic interphase and primary oocytes in meiotic prophase, especially at the stages associated with 'crossing-over' (zygotene to diplotene) which may be a hazardous process to accomplish. The steepest decline (e.g. between Days 130 and 170 gestation in the cow; Fig. 3) is linked to both atresia and the cessation of germ cell mitosis. Subsequently, when there are no oogonia left to degenerate, the atresia of oocytes settles down to a more gradual

rhythm. Rüsse (1983) ascribes the loss of most germ cells to their continuing to mature, after oocyte isolation, in an environment that is inadequate to support their development.

There is great variability between species, and between individuals within a species, as to the number of oocytes remaining at birth. The cow is born with, on average, about 235 000 oocytes, but this figure has little meaning considering that the range in a group of 69 animals was from 0 to 724 000 (Erickson, 1966b). Their numbers (as mirrored in the total number of follicles) continue to decline after birth and it is important to stress the variation from individual to individual; for example, Monniaux *et al.* (1983) found the number of follicles bigger than 70 μm in diameter to range between approximately 50 and 900 per ovary in cows. Similarly, an adult ewe may possess anywhere from 24 000 to 175 000 follicles containing oocytes, depending on the breed (Cahill *et al.*, 1979). Being far in excess of the number of oocytes or offspring that any female can produce, these numbers have sounded academic until recently. However, an understanding of the factors governing which oocytes and which follicles come to fruition is at the core of all attempts to improve yields of viable eggs either during folliculogenesis (Greve *et al.*, 1989) or during oocyte maturation *in vitro*. Study of these factors will certainly need to be extended if use is to be made of fetal oocytes.

*Folliculogenesis*

Once formed, primary oocytes *in vivo* depend for their continued development on an interaction with the female in the environment of the ovarian follicle. Use of fetal oocytes would depend on an ability to reproduce the effects of this interaction *in vitro*.

At birth, the vast majority of the thousands of primary oocytes in the ovaries are enclosed in primordial follicles, and remain so unless and until they begin to grow. in the ewe, 2–5 primordial follicles, less than 0·06 mm in diameter, enter the growth phase every day (Turnbull *et al.*, 1977; Cahill, 1982). The hormonal stimulus that causes them to do so has not been defined but presumably involves pituitary gonadotrophins because, in rats, injections of PMSG can bring it about (Lintern-Moore, 1977). Once they have entered the growth phase, sheep follicles certainly depend on the presence of gonadotrophins; this has been shown by hypophysectomy (Dufour *et al.*, 1979) and by more specific immunological neutralization of LH and FSH (McNeilly *et al.*, 1986). This dependence only becomes apparent over the long-term for the smaller, pre-antral follicles whereas a deficiency in gonadotrophins affects larger, antral, follicles much more quickly.

It takes 6 months for a primordial follicle to reach preovulatory size in sheep, of which 4–5 months are spent in growing slowly to the size at which an antrum appears and 1–2 months in a more rapid terminal phase (Cahill & Mauléon, 1980; Cahill, 1982). Even in the adult female, the large growing follicles represent only a small fraction of the total population; in the ewe, for example, only about 10% of the 200–800 growing follicles exceed 1·1 mm in diameter to become visible at the ovarian surface (Cahill *et al.*, 1979). The follicular antrum first appears in the cow in follicles ranging in diameter from 0·115 mm (when 10% possess an antrum) to 0·280 mm (by which stage 90% are antral; Monniaux *et al.*, 1983). In contrast to the oocytes of laboratory rodents, those of farm animals (pigs and cows at least) continue to grow and mature within follicles after formation of the antrum (Motlík *et al.*, 1984; Motlík & Fulka, 1986). Consequently, in pigs, oocytes isolated from small antral follicles are not usually mature enough to complete meiosis *in vitro* (Motlík *et al.*, 1984). The fact that a high proportion of bovine oocytes collected from a wide range of follicle sizes at the abattoir can be matured and fertilized *in vitro* indicates that the precise relationship between follicle size and the oocyte's meiotic competence remains to be established in cattle.

The zona pellucida, the specialized glycocalyx of the oocyte, has extremely important roles to play at the time of normal fertilization, at least, and so its formation by the oocyte as the primordial follicle starts to grow is another vital function of the primary oocyte. The growing mouse oocyte secretes at least three protein constituents of the zona pellucida which are involved in the acrosome reaction, sperm binding and the block to polyspermy. It is intriguing the the oocyte produces all of

these proteins during oogenesis and stops producing them during meiotic maturation before ovulation, yet all known functions of the products are delayed until after ovulation (Dean *et al.*, 1986).

Determining to what extent this prolonged follicular growth is essential to oocyte maturation, and to what extent its essential elements can be reproduced *in vitro*, represent formidable challenges to the possibility of using fetal oocytes and work in this area is only just beginning (Gandolfi *et al.*, 1988).

## Discussion

The usefulness of in-vitro maturation and in-vitro fertilization of follicular oocytes in cattle is beyond question. After a very prolonged developmental period, procedures have progressed enormously over the past 2 years so that zygotes, embryos and calves from in-vitro maturation and fertilization are at last available both for commercial and research purposes, as has been discussed elsewhere in this symposium. The progression of research that has led to this has involved the use of oocytes of more and more immaturity at the time of collection; only a few years ago it seemed most unlikely that large numbers of embryos would be produced from oocytes collected indiscriminately at the abattoir. Similarly, the techniques that would be necessary to make use of fetal oocytes might become feasible if there is sufficient incentive to work on them.

The advantages that in-vitro fertilization of fetal oocytes could bring to animal breeding (Tables 1 & 2) would seem real enough to encourage such work. Figure 4, in contrasting the overall principles of conventional breeding and the hypothetical scheme, illustrates another point of biological interest. Using fetal oocytes for in-vitro fertilization could eliminate the necessity for adult animals in passing from one generation to the next, with adults being siphoned off the system as required. Besides shortening the interval between generations, this would mean that the oocytes would never be exposed to the environmental influences that they encounter in the adult ovary. Some of these influences (perhaps maternal reproductive hormones, for example) could be beneficial, or even essential, to the proper maturation of the oocyte. If so, they will need to be emulated *in vitro*. Other environmental influences in the adult might be detrimental to the normal oocyte. These might include exogenous hormones used to promote growth, lactation or superovulation, physical factors such as irradiation resulting from nuclear accidents, ultrasound used for diagnostic purposes, or known toxins such as the fungicides, insecticides and polychlorinated biphenyls that have been identified in human follicular fluid (Baukloh *et al.*, 1985). Avoidance of these influences could be beneficial to animal breeding programs.

Examination of embryos recovered from normal adult donor cattle within 6 days of superovulation has shown that about 10% of them have cytogenetically detectable abnormalities. These result from abnormalities of fertilization and of oocyte maturation and ageing rather than meiotic non-disjunction (King, 1985). It might be speculated that such abnormalities could be avoided if optimal conditions for maturation and fertilization could be achieved using younger (fetal) oocytes under more controlled conditions *in vitro*. If that were to prove possible, cytogenetic abnormalities might be limited to those inevitably arising from non-disjunction due to chromosomal abnormalities already present in the germ cells.

Many biological problems will need to be overcome before fetal germ cells can be fertilized *in vitro*. The complexities of oocyte differentiation, growth and nuclear and cytoplasmic maturation have obviously evolved to permit fertilization to occur during the brief interval for which the oocyte will be exposed to spermatozoa *in vivo*. It remains to be seen which of the complexities are indispensible and which can be bypassed by improved understanding, improved culture methods and experimental manipulation. Overall, the signs seem encouraging. Mature follicles can be dispensed with in cattle. Since the early work on culturing immature mouse oocytes (see Eppig & Schroeder, 1986), it has been shown that immature follicles can be grown to antral or equivalent stages in rats (Gandolfi *et al.*, 1988; Daniel & Gore-Langton, 1988) and the contained oocytes

acquire meiotic competence and can be fertilized (abnormally) in some cases. Fetal rat ovaries can be cultured so that germ cells enter meiosis *in vitro* (Prépin *et al.*, 1985) and, in mice, primordial germ cells can be isolated from fetal ovaries and cultured *in vitro* (De Felici & McLaren, 1983; Donovan *et al.*, 1986; McCarrey *et al.*, 1987). Improved understanding of the processes controlling meiosis has led to the development of culture systems capable of maintaining mouse oocytes in a fertilizable condition for up to 40 h *in vitro* (Downs *et al.*, 1986). Primary and preantral follicles of cows can be grown in an in-vitro perifusion culture system, although the effect of such culture on the contained oocytes has not been described (Peluso & Hirschel, 1988). Sperm injection into the perivitelline space is producing pregnancies in human in-vitro fertilization programmes and so perhaps further developments in this area could reduce the need for a fully formed zona pellucida during in-vitro fertilization. Alternatively, might it be possible to obtain the genetic information from immature fetal oocytes by nuclear transplantation into enucleated mature oocytes and then using them for in-vitro fertilization? To answer these questions and many more related questions it will be necessary, as always, to integrate apparently diverse information from a variety of species, develop efficient methods of isolating fetal germ cells, understand their enormous rate of degeneration *in vivo* and prevent it *in vitro*, and give them a chance to show that a cow is only an oocyte's way of producing another oocyte.

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## References

**Baker, T.G.** (1982) Oogenesis and ovulation. In *Reproduction in Mammals*, Book 1, 2nd edn, pp 17–45. Eds C. R. Austin & R. V. Short. Cambridge University Press, Cambridge.

**Baker, T.G. & Hunter, R.H.F.** (1978) Oogenesis and follicular growth in the cow: implications for superovulation. In *Control of Reproduction in the Cow*, pp. 34–49. Ed. J. R. Sreenan. Martinus Nijhoff, The Hague.

**Baukloh, V., Bohnet, H.G., Trapp, M., Heeschen, W., Feichtinger, W. & Kemeter, P.** (1985) Biocides in human follicular fluid. *Ann. N.Y. Acad. Sci.* **442,** 240–250.

**Butler, S.** (1877) *Life and Habit*, Chapter 8. Trübner & Co., London.

**Byskov, A.G.** (1982) Primordial germ cells and regulation of meiosis. In *Reproduction in Mammals*, Book 1, 2nd edn. pp. 1–16. Eds C. R. Austin & R. V. Short. Cambridge University Press, Cambridge.

**Cahill, L.P.** (1982) Factors influencing the follicular response of animals to PMSG. In *Embryo Transfer in Cattle, Sheep and Goats*, pp. 5–7. Eds J. N. Shelton, A. O. Trounson, N. W. Moore & J. W. James. Australian Society for Reproductive Biology, Canberra.

**Cahill, L.P. & Mauléon, P.** (1980) Influences of season, cycle and breed on follicular growth rates in sheep. *J. Reprod. Fert.* **58,** 321–328.

**Cahill, L.P., Mariana, J.C. & Mauléon, P.** (1979) Total follicular populations in ewes of high and low ovulation rates. *J. Reprod. Fert.* **55,** 27–36.

**Crozet, N., Kanka, J., Motlík, J. & Fulka, J.** (1986) Nucleolar fine structure and RNA synthesis in bovine oocytes from antral follicles. *Gamete Res.* **14,** 65–73.

**Daniel, S. & Gore-Langton, R.E.** (1988) Growth and development of rat oocytes *in vitro*. *Biol. Reprod.* **38,** (Suppl. 1), 68, Abstr. 59.

**Dean, J., East, I.J. & Shimizu, S.** (1986) Biosynthesis of the mouse zona pellucida and the effect of anti-zona monoclonal antibodies on fertilization and early development. *Theriogenology* **25,** 107–116.

**De Felici, M. & McLaren, A.** (1983) *In vitro* culture of mouse primordial germ cells. *Expl Cell Res.* **144,** 417–427.

**Donovan, P.J., Stott, D., Cairns, L.A., Heasman, J. & Wylie, C.C.** (1986) Migratory and postmigratory mouse primordial germ cells behave differently in culture. *Cell* **44,** 831–838.

**Downs, S.M., Schroeder, A.C. & Eppig, J.J.** (1986) Developmental capacity of mouse oocytes following maintenance of meiotic arrest *in vitro*. *Gamete Res.* **15,** 305–316.

**Dufour, J., Cahill, L.P. & Mauléon, P.** (1979) Short- and long-term effects of hypophysectomy and ovariectomy on ovarian follicular populations. *J. Reprod. Fert.* **57,** 301–309.

**Eddy, E.M., Clark, J.M., Gong, D. & Fenderson, B.A.** (1981) Origin and migration of primordial germ cells in mammals. *Gamete Res.* **4,** 333–362.

**Eppig, J.J. & Schroeder, A.C.** (1986) Culture systems for mammalian oocyte development; progress and prospects. *Theriogenology* **25,** 97–106.

**Erickson, B.H.** (1966a) Development and radioresponse of the prenatal bovine ovary. *J. Reprod. Fert.* **11,** 97–105.

**Erickson, B.H.** (1966b) Development and senescence of the postnatal bovine ovary. *J. Anim. Sci.* **25,** 800–805.

**Gandolfi, F., Galli, C., Modina, S., Artini, P. & Lauria, A.** (1988) An in-vitro approach to the growth of ovarian follicles from primary to antral stage. *Proc. 11th Int. Congr. Anim. Reprod. & A.I., Dublin* **3,** 328a–328c.

**Greve, T. Callesen, H. & Hyttel, P.** (1989) Follicular correlates with in-vitro fertilization in cattle. *J. Reprod. Fert. Suppl.* **38,** 117–126.

**King, W.A.** (1985) Intrinsic embryonic factors that may affect survival after transfer. *Theriogenology* **23,** 161–174.

**Land, R.B. & Hill, W.G.** (1975) The possible use of superovulation and embryo transfer in cattle to increase response to selection. *Anim. Prod.* **21,** 1–12.

**Larsen, J.K., Byskov, A.G. & Christensen, I.J.** (1986) Flow cytometry and sorting of meiotic prophase cells of female rabbits. *J. Reprod. Fert.* **76,** 587–596.

**Lintern-Moore, S.** (1977) Effect of athymia on the initiation of follicular growth in the rat ovary. *Biol. Reprod.* **17,** 155–161.

**Markert, C.L. & Seidel, G.E., Jr** (1981) Parthenogenesis, identical twins and cloning in mammals. In *New Technologies in Animal Breeding*, pp. 181–200. Eds B. G. Brackett, G. E. Seidel, Jr & S. M. Seidel. Academic Press, New York.

**McCarrey, J.R., Hsu, K.C., Eddy, E.M., Klevecz, R.R. & Bolen, J.L.** (1987) Isolation of viable mouse primordial germ cells by antibody-directed flow sorting. *J. exp. Zool.* **242,** 107–111.

**McNeilly, A.S., Jonassen, J.A. & Fraser, H.M.** (1986) Suppression of follicular development after chronic LHRH immunoneutralization in the ewe. *J. Reprod. Fert.* **76,** 481–490.

**Monniaux, D., Chupin, D. & Saumande, J.** (1983) Superovulatory responses of cattle. *Theriogenology* **19,** 55–81.

**Motlík, J. & Fulka, J.** (1986) Factors affecting meiotic competence in pig oocytes. *Theriogenology* **25,** 87–96.

**Motlík, J., Crozet, N. & Fulka, J.** (1984) Meiotic competence *in vitro* of pig oocytes isolated from early antral follicles. *J. Reprod. Fert.* **72,** 323–328.

**Nicholas, F.W. & Smith, C.** (1983) Increased rates of genetic change in dairy cattle by embryo transfer and splitting. *Anim. Prod.* **36,** 341–353.

**Peluso, J.J. & Hirschel, M.D.** (1988) Factors controlling the growth of bovine primary and preautral follicles in perifusion culture. *Theriogenology* **30,** 537–546.

**Prépin, J., Gibello-Kervran, C., Charpentier, G. & Jost, A.** (1985) Number of germ cells and meiotic prophase stages in fetal rat ovaries cultured *in vitro*. *J. Reprod. Fert.* **73,** 579–583.

**Rüsse, I.** (1983) Oogenesis in cattle and sheep. *Biblthca anat.*, No. 24, 77–92.

**Surani, M.A.H., Barton, S.C. & Norris, M.L.** (1987) Experimental reconstruction of mouse eggs and embryos: an analysis of mammalian development. *Biol. Reprod.* **36,** 1–16.

**Turnbull, K.E., Braden, A.W.H. & Mattner, P.E.** (1977) The pattern of follicular growth and atresia in the ovine ovary. *Aust. J. biol. Sci.* **30,** 229–241.

**Van Raden, P.M. & Freeman, A.E.** (1985) Potential genetic gains from producing bulls with only sires as parents. *J. Dairy Sci.* **68,** 1425–1431.

**Woolliams, J.A. & Smith, C.** (1988) The value of indicator traits in the genetic improvement of dairy cattle. *Anim. Prod.* **46,** 333–345.