Low developmental capacity of *in vitro* matured and fertilized oocytes from calves compared with that of cows

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The developmental competence of oocytes from 3-month-old calves was studied through *in vitro* maturation, fertilization and culture up to the blastocyst stage and by embryo transfer into a foster mother. Oocytes were recovered from antral follicles of calves after or without ovarian stimulation with exogenous FSH and their developmental potential was compared with that of oocytes recovered from cow ovaries. Fertilization and cleavage rates from calf oocytes did not differ significantly from those of cow oocytes. However, after 7 days of culture, the blastocyst formation rate was significantly lower for calves (9% and 11% for nontreated and treated animals, respectively) than for cows (over 20%). Transfer of blastocysts obtained from calf oocytes resulted in a lower pregnancy rate (1 of 23 recipients; 4%) than that achieved with cow oocytes (10 of 26; 38%). The recipient cow that was pregnant from calf embryos delivered a full-term live calf. These data show that some key regulative event that determines the ability to form blastocysts in cattle has not been fully achieved in oocytes from 3-month-old calves.

Introduction

Oocytes from antral follicles larger than about 2 mm in diameter from several mammals are fully grown and can resume meiosis spontaneously: cows and pigs (Motlik and Fulka, 1986), sheep (Jagiello *et al.*, 1975; Szöllösi *et al.*, 1988) and goats (De Smedt *et al.*, 1994). Several lines of evidence show that the ability of these oocytes to resume meiosis depends on their hormonal environment and is associated with sequential changes in follicular steroidogenesis. Moor and Trounson (1977) showed that sheep oocytes cultured within the follicle in hormone-free medium reactivated meiosis only after addition of gonadotrophin (FSH and LH). Further experiments, using inhibitors of steroid secretion, showed that maturation of the oocytes requires a specific intrafollicular steroid environment acting synergistically with gonadotrophins (Moor *et al.*, 1980). *In vitro*, this hormonal influence appears to be beneficial not only for maturation but also for fertilization in mice (Downs *et al.*, 1986), rats (Shalgi *et al.*, 1979), sheep (Galli and Moor, 1991) and cattle (Fukushima and Fukui, 1985; Younis *et al.*, 1989). In cattle, supplementation of the maturation medium with LH and FSH and oestradiol influences both the fertilization rate and the ability of fertilized eggs to develop normally *in vitro* up to the blastocyst stage (Saeki *et al.*, 1991).

Fully grown bovine oocytes in antral follicles are present at birth. This is different from species such as mice which are widely used as an experimental model, in which oocytes from 12-day-old prepubertal females have to be grown *in vitro* before maturation and fertilization to achieve full term

development (Eppig and Schroeder, 1989). Comparing calf oocytes with cow oocytes allows examination of how the hormonal environment affects the ability of fully grown oocytes to develop following *in vitro* maturation and fertilization.

Onset of puberty corresponds to the establishment of a pulsatile pattern of LH secretion appropriate for the development of an ovulatory surge. In cattle, it is proposed that increases in progesterone play a key role in the establishment of this pattern (Schams *et al.*, 1981). A low concentration of progesterone ($< 0.1 \text{ ng ml}^{-1}$) is present from 1 to 9 months of age (Schams *et al.*, 1981) and young females become pubertal only at about 1 year of age, depending on breed and body mass. The overall mean LH and FSH concentrations decrease between 3 and 15 weeks after birth, during which time the ovaries remain exposed to a lower hormonal environment than those of cyclic cows (Dodson *et al.*, 1988).

An advantage of the postnatal bovine ovary is that the pool of growing follicles is much greater before puberty, especially between 50 and 120 days of age, than after puberty (Erickson, 1966). This explains why attempts have been made to produce embryos from young calves. Early reports by Onuma *et al.* (1970) and Seidel *et al.* (1971) indicated that female calves could respond to exogenous gonadotrophin treatment with follicular growth but that the developmental ability of oocytes was limited to two or three cleavages. However, several groups have reinvestigated the possibility of producing blastocysts from prepubertal female calves using *in vitro* maturation and fertilization. Cleavage rates higher than 50% were achieved (Palma, 1994) but data on the ability to develop further remain controversial. Armstrong *et al.* (1992) obtained a higher rate of blastocyst formation from calf oocytes than from cow oocytes (27%

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versus 17% of cleaved eggs, respectively), but only a small number of oocytes were involved in this experiment. Irvin *et al.* (1993) found that development rates for calf and cow oocytes were similar (30% versus 25%, respectively), whereas Palma *et al.* (1993) who, unlike the two previous authors, did not treat their calves with FSH, obtained a lower blastocyst rate per cleaved egg obtained from calves (16%).

The objective of the present study was to examine the developmental potential of prepubertal calf oocytes and to determine whether exogenous FSH treatment improves their developmental competence. Their ability to develop into blastocysts after *in vitro* maturation and fertilization was investigated using material from adult cows as controls. Viability of blastocysts was further assessed *in vivo* through embryo transfer. Our results show that *in vitro* development of calf oocytes to the blastocyst stage was significantly lower than that of oocytes from adults and that the further viability of such blastocysts was severely impaired.

Materials and Methods

Untreated calves

A total of 78 ovaries were collected from 39 3–4-month-old calves at the local abattoir for five replicate experiments. In each replicate, ovaries from cows were also collected (a total of 116 ovaries). Ovaries were transported to the laboratory in saline at 30–35°C and oocytes were aspirated within 2 h of slaughter from 3–6 mm follicles through an 18 gauge needle under constant vacuum.

Oocytes surrounded by more than three compact layers of cumulus cells were selected, washed and submitted to *in vitro* maturation and fertilization as described by Mermillod *et al.* (1992). Briefly, oocytes were matured for 24 h in pools of about 100 in TCM199 (Gibco, Paisley) containing 10% (v/v) heat-treated fetal calf serum, 1 µg oestradiol ml⁻¹ (Sigma, St Louis, MO), 5 µg pLH ml⁻¹ and 0.5 µg pFSH ml⁻¹ (pure pLH and pFSH were provided by J. F. Beckers, Liège). Spermatozoa were prepared on Percoll (Pharmacia, Uppsala) gradients and fertilization was carried out in 4-well tissue culture plates for 18 h at 39°C under 5% CO₂. Each well contained about 100 oocytes and 10⁶ spermatozoa in 500 µl TALP supplemented with 10 µg heparin ml⁻¹ (Na salt, 167 U mg⁻¹, Calbiochem, San Diego, CA). After *in vitro* fertilization, oocytes were cleaned of surrounding cumulus cells by vortex agitation for 1 min in PBS. Cow and calf oocytes were randomly selected from each replicate and fixed in ethanol after maturation and fertilization, and stained with Hoechst 33342 fluorescent dye to evaluate the fertilization rate. This rate was evaluated as the ratio of number of cleaved eggs + noncleaved eggs with two pronuclei:total number of inseminated oocytes. Remaining presumptive zygotes were cultured (39°C, 5% CO₂ in air) for 7 days in mineral oil overlaid with drops (1 µl per embryo) of serum free TCM199 previously conditioned on bovine oviduct epithelial cell monolayers (Mermillod *et al.*, 1993). Cleavage was recorded on day 2 of culture (3 days after insemination) and blastocyst rate on day 7 (8 days after insemination).

Treated calves

A total of 14 Holstein calves were treated at 3 months of age. Treatment was adapted from Saumande and Chupin (1994), using a combination of a progestagen implant (Norgestomet, Intervet, Angers) inserted s.c. for 4.5 days, and 8 i.m. injections of pFSH given at intervals of 12 h with a total dose equivalent to 16 mg (NIH-FSH units) (Stimufol, Rhône-Merieux). Animals were killed 12 h after the final pFSH injection at the experimental abattoir of the institute and the ovaries were immediately recovered and transferred to the laboratory.

Control immature oocytes were recovered from ovaries of cows in a local abattoir and subjected to *in vitro* maturation and fertilization under the same conditions as the calf oocytes. As soon as ovaries arrived in the laboratory, cumulus–oocyte complexes were collected by aspiration from follicles over 3 mm diameter present at the surface of the ovary of calves or cows. The cumulus–oocyte complexes of calves were allocated to two groups according to the size of follicles they originated from: either small (3–8 mm) or large (> 8 mm) follicles. After washing three times in TCM199 and morphological evaluation, intact cumulus–oocyte complexes, with several dense cumulus cell layers from calf or control cow ovaries were selected for *in vitro* maturation. This was performed in TCM199 supplemented with 10% (v/v) fetal calf serum, 10 µg FSH ml⁻¹ and 1 µg LH ml⁻¹ and 1 µg oestradiol ml⁻¹ for 24 h at 39°C in a humidified atmosphere of 5% CO₂ and air on a bovine granulosa cell monolayer. At the end of the maturation period, selected cumulus–oocyte complexes were inseminated using the *in vitro* fertilization as described by Marquant-Le Guenne *et al.* (1990). Briefly, frozen–thawed sperm cells, separated by the swim up technique and heparin capacitated, were used for insemination at a concentration of 1 × 10⁶ cells ml⁻¹. Eighteen hours later, the eggs were rinsed in Hepes-buffered TCM199 and dechorionized by pipetting before culturing. For each replicate experiment, zygotes from calf and cow oocytes were co-cultured for 7 days at 39°C on bovine oviductal epithelial cell monolayers prepared by primoculture 3–5 days before the start of the culture in 4-well dishes (Nunc/Clon-Delta, Roskilde). The cleavage rate was evaluated on day 2 (3 days after insemination). Noncleaved embryos were fixed in acetic alcohol, stained with aceto–orcein and observed under the microscope to determine the presence of two pronuclei to evaluate the fertilization rate.

On day 7 of co-culture, development was assessed by evaluating the proportion of blastocysts formed from cow and calf oocytes.

Viability assessment of the *in vitro* derived blastocysts

Some of the blastocysts that had developed *in vitro* from oocytes of nontreated or FSH-treated calves and from cow oocytes were transferred to recipient heifers to assess their viability. The calf and cow blastocysts used for transfer were produced during the same series of experiments. One or two blastocysts were non-surgically transferred to each recipient on day 7 of the oestrous cycle. After transfer, each recipient was checked twice daily for return to oestrus. A blood sample was taken by day 21 for a rapid plasma progesterone assay and estimation of initiated pregnancies. Fetal development was

Table 1. *In vitro* development of oocytes from untreated calves after *in vitro* maturation and fertilization, and comparison with cow oocytes

Origin of cumulus-oocyte complex	Oocytes recovered and matured [Replicates]	Oocytes inseminated and cultured	Rates of		
			Fertilization	Cleavage	Blastocyst formation
Untreated calves (<i>n</i> = 39) [Range]	750 [5]	662	72% ^a (21/29)	81% ^a (510/663) [76%–90%]	9% ^a (55/633) [2%–16%]
Cows (Control) (<i>n</i> = 44) [Range]	560 [5]	496	62% ^a (15/24)	85% ^a (402/472) [81%–89%]	21% ^b (97/472) [12%–28%]

Chi-square test: percentages with different letters within the same column differ significantly ($P < 0.05$).

first diagnosed by ultrasonography on some of the recipients on day 35 and pregnancies were then confirmed by rectal palpation after 3 months.

Statistical analyses

Percentages of fertilization, cleavage and blastocyst formation in each group of calf oocytes were first compared with those of control cow oocytes by means of chi-square analysis.

In addition, to take into account the possible existence of a replication effect and of an interaction between replicates and age, a generalized linear model (with a binomial distribution and a logit link, McCullagh and Nedler, 1989) was used. This application of likelihood ratio tests checks the validity of this model as an additive one. If the model is found to be invalid, this means that there is a significant interaction between replicates and age concerning the rate of blastocyst formation.

Results on the development of embryos *in vitro* from treated and nontreated calves were presented separately since each experiment was conducted with its own control.

Results

Rate of blastocyst formation from oocytes of untreated calves

A mean of 19 cumulus-oocyte complexes per calf was recovered from the 39 calves and a total of 662 cumulus oocyte complexes were matured *in vitro* and inseminated. They were cultured in parallel to 496 control cumulus-oocyte complexes from ovaries of 44 cows in five replicate experiments. The abilities of calf and cow oocytes to be fertilized, to cleave, and to develop to the blastocyst stage were compared (Table 1).

The fertilization and cleavage rates of the oocytes from calves did not differ from those of cows (72% versus 62% and 81% versus 85%, respectively). However, after 7 days in culture, the rate of blastocyst formation was lower for the calf than for the cow zygotes (9% versus 21%). No interaction between replication and age effects could be found and the rate of blastocyst formation was found to be significantly lower ($P < 0.001$) for calf than for cow oocytes.

Rate of blastocyst formation from oocytes of FSH-treated calves

Treatment of 14 calves with FSH, followed by aspiration of follicles, resulted in an average of 39 ± 22 cumulus-oocyte complexes per animal; 79% of them were morphologically intact. These were used for *in vitro* maturation and fertilization simultaneously with 278 control cumulus-oocyte complexes from ovaries of 23 cows in nine replicate experiments (Table 2).

Calf oocytes were fertilized and cleaved *in vitro* in the same proportion as those from cows (fertilization rate 85% and 87% and cleavage rate 73% and 79%, respectively). However, after 7 days of *in vitro* culture, a smaller proportion of blastocysts developed from calf than from cow oocytes (11% versus 27%; $P < 0.05$). There was evidence of an interaction between replication and age effects but the additive model remained valid for five of nine replicates and, in those cases, the rate of blastocyst formation was found to be significantly lower ($P < 0.001$) for calf than for cow oocytes.

With FSH treated calves, a mean of 3.3 blastocysts was obtained per treated calf while only 1.4 blastocysts were obtained per nontreated calf. This is a consequence of the greater number of cumulus-oocyte complexes recovered (39 cumulus-oocyte complexes for the FSH-treated versus 19 for the untreated calves) as developmental ability to the blastocyst stage did not differ between oocytes of the two groups (9% for nontreated and 11% for FSH-treated calves, respectively). The number of blastocysts developed *in vitro* from individual treated calves was highly variable. About 15% of the calves produced no blastocyst, while 30% produced four and another 20% produced more than six blastocysts. This was a consequence of the variation in number of oocytes recovered from individual animals, which ranged from 10 to 78.

No marked differences were observed in the fertilization and cleavage rates of calf oocytes collected from small or large follicles (85% versus 86% and 70% versus 76%, respectively) (Fig. 1). However, after 7 days *in vitro*, there was 6% blastocyst formation of oocytes from small follicles compared with 16% in those originating from large follicles ($P < 0.05$). In both cases, developmental ability remained significantly lower than that of cow oocytes (27%; see Table 2).

Table 2. *In vitro* development of oocytes from FSH-treated calves after *in vitro* maturation and fertilization, and comparison with cow oocytes

Origin of cumulus-oocyte complex	Oocytes recovered and matured [Replicates]	Oocytes inseminated and cultured	Rates of		
			Fertilization	Cleavage	Blastocyst formation
FSH treated calves (<i>n</i> = 14) [Range]	554 [9]	438	85% ^a (374/438) [72%–95%]	73% ^a (320/438) [60%–83%]	11% ^a (46/438) [0%–43%]
Cows (<i>n</i> = 23) [Range]	278 [9]	278	87% ^a (243/278) [66%–100%]	79% ^a (221/278) [88%–96%]	27% ^b (75/278) [10%–44%]

Chi-square test: percentages with different letters within the same column are significantly different ($P < 0.05$).

Fig. 1. *In vitro* development of oocytes from FSH-treated calves according to follicle size. (□) Calf cumulus-oocyte complexes from follicles 3–8 mm (*n* = 247). (■) Calf cumulus-oocyte complexes from follicles > 8 mm (*n* = 191). Fertilization rate, cleavage rate and blastocyst rate are related to the number of oocytes inseminated. Statistical notation (*) is a comparison by means of chi-square analysis of the blastocyst rate of calf oocytes collected from small follicles (3–8 mm), with that of calf oocytes collected from large follicles (> 8 mm), * ($P < 0.05$).

Survival *in vivo* of blastocysts produced *in vitro*

Fourteen blastocysts from untreated calves were transferred into 14 recipients (one per recipient); 18 blastocysts from FSH treated calves were transferred into nine recipients (two per recipient); and 26 blastocysts from control cows were transferred into 26 recipients (one per recipient). Rates of initiated pregnancies were 36%, 66% and 65%, respectively. However, only one of nine recipients (11%) transferred with embryos from treated calves maintained pregnancy and delivered a full-term male calf weighing 45 kg at birth. This rate is much lower than that obtained after transfer of *in vitro* matured and fertilized cow blastocysts (38%). This indicates a higher embryonic or fetal mortality for calf blastocysts than for cow blastocysts developed and transferred under the same conditions (Table 3).

Discussion

The results reported here show that the developmental ability of embryos obtained *in vitro* from 3-month-old treated or nontreated calves is significantly lower than that of embryos obtained from cows. This lower developmental ability of calf oocytes has also been reported by Kajihara *et al.* (1991) and Palma *et al.* (1993), but differs from results reported by Armstrong *et al.* (1992) and Irvin *et al.* (1993). The last two authors observed a higher developmental ability of calf oocytes to the blastocyst stage, but their experiments involved a limited number of oocytes, recovered from young (3–9 weeks of age) FSH-treated calves.

The lower developmental ability of oocytes from 3-month-old calves compared with that of cyclic cow oocytes shown here may depend on some defective endocrine environment encountered *in vivo* before the onset of puberty. In cattle, the mean LH and FSH concentrations decrease between 3 and 15 weeks of age (Dodson *et al.*, 1988); therefore the 12-week-old calves used in these experiments probably had lower concentrations of gonadotrophins at the time of oocyte removal.

Hormonal pretreatment of the calves, which combined a progestagen implant and FSH administration, did not improve the developmental ability of the resulting embryos. After stimulation, oocytes from large follicles (> 8 mm) developed better than did those from small follicles (3–8 mm), as has been observed with cow ovaries (Pavlok *et al.*, 1992; Lonerghan *et al.*, 1994), in which there is a relationship between oocyte competence to form a blastocyst and the size of the antral follicle it originates from. Nevertheless, the mean rate of blastocyst formation in calves was always significantly lower than that in cows. Thus, the superovulatory treatment, which is known to stimulate the growth of 2–3 mm antral follicles (Testart, 1972), resulted in an increased number of available oocytes, but was associated with a higher variability of response between animals. Such variability (ranging from 10 to 78 oocytes recovered per animal) has also been observed after PMSG injection (Onuma *et al.*, 1970; Seidel *et al.*, 1971).

In the study reported here, the lower developmental ability of calf oocytes was observed only from the blastocyst stage onwards. The *in vitro* fertilization and cleavage rates did not differ from those obtained with oocytes from adult females, but the proportion of morphologically normal blastocysts formed

Table 3. *In vivo* survival after transfer of blastocysts produced *in vitro* from calf and cow oocytes

Source of IVF derived blastocysts	Recipients [Blastocysts]	Pregnancies		
		Initiated Day 21 (%)	Lost (%)	Confirmed > 6 month (%)
Untreated calves	14 [14]	5/14 (36%)	5/5 (100%)	0/14 (0%)
FSH-treated calves	9 [18]	6/9 (66%)	5/6 (83%)	1/9 (11%)
Control cow oocytes	26 [26]	17/26 (65%)	7/17 (41%)	10/26 (38%)

was less than half that obtained from cows. The blastocysts derived from calves had a visible inner cell mass and could not be distinguished from those of cows by size or by timing of formation. However, their competence to develop further following transfer to a recipient foster mother was severely limited. Only one recipient of 23 (4%) maintained pregnancy until calving and delivered a full-term live calf, despite a normal rate of initiated pregnancies by day 21 (48%), as assessed by progesterone assay. Pregnancy losses after transfer of blastocysts from calves were more than twice as frequent as losses after transfer of blastocysts from control cows. To our knowledge, there have been only two reports of birth after transfer of embryos from calf oocytes. Armstrong *et al.* (1992) obtained one calf but, in this case, the transferred blastocysts were derived from oocytes matured *in vivo* and removed from 5–6-week-old calves. Kajihara *et al.* (1991) reported the birth of one calf from a blastocyst derived *in vitro* from oocytes of 4-month-old superovulated calves.

The data presented here have several implications. First, the low developmental ability that characterizes calf oocytes underlines the importance of the follicular microenvironment before puberty. Stimulation of follicular growth by exogenous gonadotrophins for 4 days did not produce in fully grown oocytes the developmental competence that occurs after the onset of puberty. Puberty is not only characterized by the establishment of a pulsatile pattern of LH secretion, appropriate for the final development of the ovulatory follicle (Schams *et al.*, 1981), but also by a long period of gonadal maturation. Even if the gonads have differentiated enough to respond to an exogenous hormonal stimulation, the number of specific gonadotrophin receptors on target cells may be insufficient (reviewed by Levasseur, 1979). Cell signalling between the oocyte and the surrounding cells within the follicle depends on the presence of these receptors, which make the oocyte more sensitive to its hormonal microenvironment. These maturation events have not occurred in calf oocytes, and this would explain their poor developmental competence.

Second, it appears that some key regulation, which determines the ability to form blastocysts in adult cows, has not been fully achieved in oocytes from 3-month-old calves, even in follicles over 3 mm in diameter. This 'maternal effect' may also concern the further developmental ability of these blastocysts, and suggests a long-lasting effect of follicular oocyte components on embryonic development. The maternal effect

may be related to the presence of an abnormal protein profile observed by Lévesque and Sirard (1994) in untreated calf oocytes, and may arise from qualitative modification in the mRNA population of these oocytes at the beginning of folliculogenesis. The rate of RNA synthesis is high in oocytes of small antral follicles (Motlik *et al.*, 1984; Motlik and Fulka, 1986; Moor and Gandolfi, 1987) and this is necessary for the storage of information (RNAs or proteins) essential not only for resumption of meiosis but also for early embryonic development (Moor and Powell, 1989; Sirard *et al.*, 1992). This finding suggests that some rate-limiting factor(s) of the oocyte is (are) involved in reducing the developmental ability of embryos obtained from female calves. Attempts to identify these factors would require a molecular analysis of groups of oocytes in the same physiological state. If these factors are involved at the mRNA level, then methods are now available for differential screening from a reduced number of cells (Smith and Gridley, 1992; F. Revel, P. Mermillod, N. Peynot, J. P. Renard and Y. Heyman, unpublished).

Finally, these results indicate that caution is required when considering the possibility of using calves for reducing the generation interval in breeding schemes. Whether the low developmental ability observed with oocytes obtained from 3-month-old calves after *in vitro* maturation and fertilization and embryo transfer will also be encountered in older or even younger animals, remains to be determined. In fact, recent data show that with younger animals (5-week-old calves) a high percentage (30%) of blastocysts can be obtained (Armstrong *et al.*, 1994). It is clear that the concept of velogenesis, proposed by Georges (1991), for reducing the generation interval in cattle by using fetal oocytes, needs further experimental confirmation. The feasibility of such an approach has been shown, with limited success, in mice (Eppig and Schroeder, 1989; Carroll *et al.*, 1990), in which oocytes grow for only 15 days (Schultz, 1986). In cattle, oocyte growth lasts at least five times as long (Betteridge *et al.*, 1989) and has not yet been achieved *in vitro*. This finding, together with the results presented here of experiments using fully grown *in vivo* oocytes, makes the attractive idea of velogenesis probably a long-term goal.

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References

- Armstrong DT, Holm P, Irvin B, Petersen BA, Stubbings RB, McLean D, Stevens G and Seamark RF (1992) Pregnancies and live birth from *in vitro* fertilization of calf oocytes collected by laparoscopic follicular aspiration *Theriogenology* **38** 667–678
- Armstrong DT, Irvin B, Earl C, McLean D, Stevens G and Seamark RF (1994) Gonadotropin stimulation regimens for follicular aspiration and *in vitro* embryo production from calf oocytes *Theriogenology* **42** 1227–1236
- Betteridge KJ, Smith C, Stubbings RB, Xu KP and King WA (1989) Potential genetic improvement of cattle by fertilization of fetal oocytes *in vitro* *Journal of Reproduction and Fertility Supplement* **38** 87–98
- Carroll J, Whittingham DG, Wood MJ, Telfer E and Gosden RG (1990) Extra-ovarian production of mature viable mouse oocytes from primary follicles *Journal of Reproduction and Fertility* **90** 321–327
- De Smedt V, Crozet N and Gall L (1994) Morphological and functional changes accompanying the activation of meiotic competence in ovarian goat oocytes *Journal of Experimental Zoology* **269** 128–139
- Dodson SE, McLeod BJ, Haresign W, Peters AR and Lamming GE (1988) Endocrine changes from birth to puberty in the heifer *Journal of Reproduction and Fertility* **82** 527–538
- Downs SM, Schroeder AC and Eppig J (1986) Developmental capacity of mouse oocytes following maintenance of meiotic arrest *in vitro* *Gameete Research* **15** 305–316
- Eppig JJ and Schroeder AC (1989) Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation and fertilization *in vitro* *Biology of Reproduction* **41** 268–276
- Erickson BH (1966) Development and senescence of the postnatal bovine ovary *Journal of Animal Science* **25** 800–805
- Fukushima M and Fukui Y (1985) Effects of gonadotropins and steroids on the subsequent fertilizability of extrafollicular bovine oocytes cultured *in vitro* *Animal Reproduction Science* **9** 323–332
- Galli C and Moor RM (1991) Gonadotrophin requirements for the *in vitro* maturation of sheep oocytes and their subsequent embryonic development *Theriogenology* **35** 1083–1093
- Georges M (1991) Perspectives for marker-assisted selection and velogenetics in animal breeding. In *Animal Application of Research in Mammalian Development* pp 285–325 Eds RA Pedersen, A McLaren and NL First. Cold Spring Harbor Laboratory Press, New York
- Irvin B, Armstrong DT, Earl C, McLean D and Seamark RF (1993) Follicle development and oocyte recovery from calves with repeated gonadotropin stimulation and follicular aspiration *Theriogenology* **39** 237 (Abstract)
- Jagiello G, Mercedes D, Miller W, Graffeo J and Fang JS (1975) Stimulation and inhibition with LH and other hormones of female mammalian meiosis *in vitro* *Journal of Reproduction and Fertility* **43** 9–22
- Kajihara Y, Blakewood EG, Myers MW, Kometani N, Goto K and Godke RA (1991) *In vitro* maturation and fertilization of follicular oocytes obtained from calves *Theriogenology* **35** 220 (Abstract)
- Levasseur MC (1979) Thoughts on puberty: the gonads *Annales de Biologie Animale, Biochimie et Biophysique* **19** 321–335
- Lévesque JT and Sirard MA (1994) Proteins in oocytes from calves and adult cows before maturation: relationship with their development capacity *Reproduction Nutrition Développement* **34** 133–139
- Lonerghan P, Monaghan P, Rizo D, Boland MP and Gordon I (1994) Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization and co-culture *in vitro* *Molecular Reproduction and Development* **37** 48–53
- McCullagh P and Nedler JA (1989) Generalized linear models. In *Monographs on Statistics and Applied Probability* pp 1–511. Chapman and Hall, London
- Marquant-Le Guenne B, Humblot P, Thibier M and Thibault C (1990) Evaluation of bull semen fertility by homologous *in vitro* fertilization tests *Reproduction Nutrition Développement* **30** 259–266
- Mermillod P, Massip A and Dessy F (1992) *In vitro* production of cattle embryos: review and Belgian results *International Journal of Developmental Biology* **36** 185–195
- Mermillod P, Vansteenbrugge A, Wils C, Mourmeaux JL, Massip A and Dessy F (1993) Characterization of the embryotropic activity of exogenous protein-free oviduct-conditioned medium used in culture of cattle embryos *Biology of Reproduction* **49** 582–587
- Moor RM and Gandolfi F (1987) Molecular and cellular changes associated with maturation and early development of sheep eggs *Journal of Reproduction and Fertility Supplement* **34** 55–69
- Moor RM and Powell DJ (1989) Translational capacity of sheep oocytes microinjected with messenger RNA *Journal of Reproduction and Fertility* **86** 289–295
- Moor RM and Trounson AO (1977) Hormonal and follicular factors affecting maturation of sheep oocytes *in vitro* and their subsequent developmental capacity *Journal of Reproduction and Fertility* **49** 101–109
- Moor RM, Polge C and Willadsen SM (1980) Effect of follicular steroids on the maturation and fertilization of mammalian oocytes *Journal of Embryology and Experimental Morphology* **56** 319–335
- Motlik J and Fulka J (1986) Factors affecting meiotic competence in pig oocytes *Theriogenology* **25** 87–96
- Motlik J, Kopečný V, Trávník P and Pivko J (1984) RNA synthesis in pig follicular oocytes: autoradiographic and cytochemical study *Biology of the Cell* **50** 229–236
- Onuma H, Hahn J and Foote H (1970) Factors affecting superovulation, fertilization and recovery of superovulated ova in prepubertal cattle *Journal of Reproduction and Fertility* **21** 119–126
- Palma GA (1994) Effect of FSH and estradiol-17 β for maturation of calf oocytes on the *in vitro* development to blastocysts *Theriogenology* **41** 267 (Abstract)
- Palma GA, Clement-Sengevald A and Krefft H (1993) *In vitro* production of cattle embryos from calf oocytes *Theriogenology* **39** 278 (Abstract)
- Pavlok A, Lucas-Hahn A and Niemann H (1992) Fertilization and developmental competence of bovine oocytes derived from different categories of antral follicles *Molecular Reproduction and Development* **31** 63–67
- Saeiki K, Hoshi M, Leibried-Rutledge ML and First NL (1991) *In vitro* fertilization and development of bovine oocytes matured in serum-free medium *Biology of Reproduction* **44** 256–260
- Saumande J and Chupin D (1994) Ovarian stimulation in prepubertal heifers *Proceedings 10th Scientific Meeting of European Embryo Transfer Association* p 244 (Abstract)
- Schams D, Schallenberger E, Gombe S and Karg H (1981) Endocrine patterns associated with puberty in male and female cattle *Journal of Reproduction and Fertility Supplement* **30** 103–110
- Schultz R (1986) Molecular aspects of mammalian oocyte growth and maturation. In *Experimental Approaches to Mammalian Embryonic Development* pp 195–217 Eds J Rossant and RA Pedersen. Cambridge University Press, Cambridge
- Seidel GE, Larson LL and Foote RH (1971) Effects of age and gonadotropin treatment on superovulation in the calf *Journal of Animal Science* **33** 617–622
- Shalgi R, Dekel N and Kraicer PF (1979) The effect of LH on fertilization and developmental capacity of rat oocytes matured *in vitro* *Journal of Reproduction and Fertility* **55** 429–435
- Sirard MA, Coenen K and Bilodeau S (1992) Effect of fresh or cultured follicular fraction on meiotic resumption in bovine oocytes *Theriogenology* **37** 39–57
- Smith D and Gridley T (1992) Differential screening of a PCR-generated mouse embryo cDNA library: glucose transporters are differentially expressed in early postimplantation mouse embryos *Development* **116** 555–561
- Szölösi D, Desmedt V, Crozet N and Brender C (1988) *In vitro* maturation of sheep ovarian oocytes *Reproduction Nutrition Développement* **28** 1047–1080
- Testart J (1972) Réponse folliculaire du veau femelle impubère a divers traitements de gonadotropine sérique associée ou non a un progestagène *Annales de Biologie Animale, Biochimie et Biophysique* **12** 397–409
- Younis AI, Brackett BG and Fayer-Hosken RA (1989) Influence of serum and hormones on bovine oocytes maturation and fertilization *in vitro* *Gameete Research* **23** 189–201