Reviews

Mouse and bovine models for human IVF*

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Abstract

It is obvious that the first prerequisite is to define for what purpose a model is needed for humans. There are huge differences in reproductive physiology between the mouse, human and cow. As far as maturation is concerned, the plasticity of the mouse model is not the same in cows and humans. The final stages of oocyte maturation seem to be more finely regulated in cows and humans, where a minimum size of follicle is necessary to complete maturation *in vitro*. Bovine and human preimplantation embryos seem to be more similar in terms of biochemical and intrinsic paternal and maternal regulatory processes. Once again, interactions between the embryo and the corpus luteum are similar in cows and humans, but mouse and human embryo implantations are closer. Mouse oocytes and embryos should not be overlooked, but excessive generalization between mammalian species must be avoided.

Keywords: *bovine, embryos, human, implantation, mouse, oocytes*

Introduction

When talking of a model, it is necessary to define the purpose of that model. The mouse model is the most useful and popular model for embryo manipulation. It has been studied as a model for understanding biochemical and physiological regulations of mammalian embryo in general. Its popularity explains why, to date, many of the processes involved in embryo fertilization have been successfully reproduced *in vitro*. It can be proposed as a 'pharmacotoxicological tool' to determine the suitability of the methods, techniques and materials used in human IVF.

In-vitro maturation from preantral follicles, followed by fertilization and birth of offspring, has, for example, only been successful in the mouse. All mouse physiological processes during the life cycle are accelerated (**Table 1**). Puberty begins a few weeks after birth, follicle growth and final stages of maturation take 48 h, and the sexual cycle is short in comparison with that in humans. In-vitro experiments, always the most difficult, can be shortened, increasing the likelihood of success. The 'physiological' differences are considerable: the mouse is a polyovulatory animal where embryonic signalling is not clear and maintenance of corpus luteum function depends on totally different regulation. This does not mean that the mouse model must be overlooked; it is a cheap resource, and is necessary for first approaches in embryology, i.e. embryo manipulation.

In this review, an attempt will be made to determine which parameters make the mouse a good model for human studies. An explanation will then be advanced for why the bovine model can generally provide more information.

Oocyte maturation

There are important species differences in regulation and timing of maturation, which makes it difficult to transpose techniques. Only in the mouse model have live pups been born from primordial or early preantral follicles cultured entirely *in vitro*. In humans, clinical results are still weak (for review see Cortvrindt and Smitz, 2001), even if some progress has been made recently (Mikkelsen *et al.*, 2000; Trounson *et al.*, 2001). The follicle must attain a certain minimum size in order to complete oocyte maturation *in vitro*, and the human situation is similar to that observed in sheep and cows.

During the initial stages of zygote formation and early cleavage divisions, there is a minimal level of transcription

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only (human: Ao *et al.*, 1994; mouse: Aoki *et al.*, 1997; cow: Memili and First, 1999). For this aspect of very early embryogenesis, the mouse model is the only one available, due to its low cost, although there is no evidence for similarity with the other mammalian models.

At the time of ovulation, the mature oocyte must contain a storage pool of proteins and/or mRNA transcripts. Early embryonic development will proceed under the control of these maternal stores. This stored pool must be capable of coding for all the enzymes required for metabolic pathways, at the correct equilibrium to support the early stages of embryo development prior to activation of the zygote genome.

The cycle during which the zygote genome is activated (zygotic gene activation, ZGA) is the longest cycle of preimplantation development: any delay at this time will result in a decrease in the concentration of mRNA below critical thresholds. Polyadenylation is a regulatory process allowing control of the stability of the mRNAs and their possibility of being translated. Polyadenylated mRNA is ready for translation but it is also more susceptible to degradation. Polyadenylation is mediated via an enzyme, polyadenylate polymerase (PAP); to add further complexity to this regulation, the mRNA coding for PAP is also susceptible to polyadenylation.

Important modifications may occur during the very final stages of oocytes maturation between the germinal vesicle (GV) stages and metaphase 2 (MII) (**Table 2**). We have tested several mRNAs coding for enzymes involved in protection against free radicals and/or reactive oxygen species and transport of metabolites (MCT). Species differences arise in polyadenylation. All the mRNAs we have tested seem to be ready for translation in the mouse, even at the GV stage. This is not the case in humans and cows, where polyadenylation is more finely regulated. In our preliminary experiments on oocyte maturation collected in intracytoplasmic sperm injection (ICSI) patients, we found that only one out of seven matured oocytes had the correct polyadenylation profile, even if the MII stage was reached in time (El Mouatassim *et al.*, 1999).

Some mRNAs need to be active up to blastocyst formation. Stability does not seem to be a problem in the mouse, where again all processes are shorter. The cycle of genomic activation is between two- and four-cell stages so there is no need for a balance between stability and efficiency. The consequence for embryo culture in our experiments is that the embryo is protected against negative impacts of culture conditions. In cows and humans, a balance between stability and readiness for translation has to be maintained.

Table 1. Some parameters of mouse, human and cattle oocytes and embryos (from Betteridge and Rieger 1993).

	Human	*Cow*	*Mouse*
Oocyte diameter[a] (µm)	150–180	150–180	90–100
Time (h) to reach			
2-cell stage	30	36	12
Blastocyst	120	150	70
Hatching	150	200	100
Stage of ZGA	4-C	8-C	2-C

[a]The volume ratio human/mouse oocyte is 2.3.

Table 2. Regulation of mRNA polyadenylation during final stages of oocyte maturation in mouse and human embryos. From El Mouatassim *et al.* (1999) and Hérubel *et al.* (2002).

	Human		*Mouse*	
	VG	MII	VG	MII
Baseline	–	+	–	+
GCS	+	+	+	+
GPX	–	+	+	+
Cu-Zn SOD	+	+	+	+
Mn SOD	–	+	+	+
Catalase	–	–	–	–

+ = PolyA tail present.

Fertilization and parthenogenesis

Most of the specific events related to fertilization have been described in the mouse, and are valid for other species. In all animal species, fertilization takes place spontaneously in simple media buffered with bicarbonate and containing serum albumin and glucose. In mice, epididymal spermatozoa are used, but this does not seem to make much difference. The preparation of spermatozoa for IVF in domestic animals is very similar to that used for human. A temperature of 39°C has an important impact on success in cows. For both cows and humans, this success has a paternal origin (see below under 'Intrinsic factors in the regulation of embryo development').

The mouse oocyte can be used for training, i.e. the manipulations used in ICSI, since its ooplasm is clear. This is not the case for porcine and bovine ooplasm. Technically speaking, mouse spermatozoa are not suitable for ICSI manipulation because of the large size of the acrosome. For studies on parthenogenesis, the mouse is obviously not a good model, and the mouse centrosome is derived through maternal inheritance. Complementarity of parental genomes has been described in the mouse (MacGrath and Solter, 1984) and is valid for all other species.

Embryo metabolism co-culture and culture

Biophysical parameters: the effect of O_2 concentration in the gas phase

In the mouse, as for the human embryo, a 5% O_2 concentration does not dramatically improve embryonic growth. In cows, sequential media (SOF, ISM) can be used only with 5% O_2, contrary to co-culture with Vero cells where 5% CO_2 in air works well. Reduced osmolarity, believed to favour rapid RNA synthesis in mice (Ho *et al.*, 1994), has been proposed for human embryo culture, and some commercial preparations may have an osmolarity around 260 mOsm/kg. However, neither in humans nor in cows has any real improvement been observed.

It is important to determine, if the mouse model can be used, and if so, to define the strain (and especially the value of

outbred and hybrid strains) and the development stage of the embryo. There are some obvious points to be outlined here: outbred mouse embryos are more difficult to grow from the 1-cell stage. From the 2-cell stage they are very easy to grow in every kind of medium, so that no discriminant effect is observed with this type of culture. Mouse embryos were used in the initial studies on cytoplasmic transfer and prevention of developmental arrests (Muggleton-Harris *et al.*, 1982), but produced no valid information useful for human IVF.

Developmental arrest, when growing 2-cell stage embryos collected *in vitro*, implies high toxicity or improper methods of manipulation. An embryo collected *in vivo* at this stage or immediately after does not offer any difficulties in culture. The crucial point is growth before or after zygotic gene activation (ZGA).

As mentioned earlier, the stored pool (mRNA and proteins) must be theoretically capable of coding for all the enzymes required for metabolic pathways, but this is hardly possible. Moreover, the enzyme may have to cope with incorrect equilibrium conditions (Ménézo and Khatchadourian, 1990). Referring back to the models, one of the pioneering studies was carried out by Chi *et al.* (1988). A comparison of the concentrations of some enzymes between human and mouse embryos showed that concentrations may vary easily by 30-fold. So, when offered the same substrates, the biochemical machinery does not work at the same speed in the two species, and it is common knowledge that the slowest enzymatic activities make the rule.

Embryo metabolism and the so-called toxicity of glucose

This situation depends on various genetic aspects of storage in the oocytes. Glucose can be toxic or non-toxic in different strains of inbred mice. A few enzymes, in relation or not with O_2 concentration, can be involved in this situation, including glucose phosphate isomerase and phosphofructokinase (Barbehen *et al.* 1974; Ménézo and Khatchadourian, 1990). Once more, some strains possess this kind of feature but others do not. Replacement of glucose by fructose can be useful or not, depending upon the strain.

It is also clear, from human data, that the absence of glucose utilization may depend on a critical low threshold of hexokinase (El Mouatassim *et al.*, 1998). The bovine embryo seems to resemble more closely the human rather than the mouse embryo. It can be grown with glucose from the 1-cell stage onwards if the O_2 concentration is decreased to 5%. High O_2 concentrations may seriously affect metabolism, whatever the embryo.

pH regulation and monocarboxylic acid transporters

Cells regulate pHi through exchangers on the plasma membrane. They include a $Na^+/HCO_3^-/Cl^-$ exchanger and a Na/H exchanger, which increase the pH and HCO_3/Cl to decrease the pHi. Early stage embryos can operate in an alkaline pH but not in an acidic one. Another example of different regulation between embryos arises with H^+-monocarboxylate co-transport (Gibb *et al.*, 1997). There are four major monocarboxylate transporters (MCT). The most striking difference between human and mouse MCT expression concerns MCT4 transcripts: in humans, these transcripts are barely detectable in pools of oocytes or embryos, suggesting a weak biological influence during early embryonic development. In contrast, MCT4 mRNAs are easily detectable in single mouse oocytes or embryos. This difference between humans and mice can explain differences in embryonic pHi regulation. Indeed, early human embryos up to the morula/blastocyst transition are unable to recover from transitory acidosis (Dale *et al.*, 1998), whereas early mouse embryos can regulate acidosis through H^+-monocarboxylate co-transport (Gibb *et al.*, 1997). Besides, MCT4 is a low affinity transporter, with high capacity and preference for lactate, making it adaptable for lactate release (Dimmer *et al.*, 2000). MCT4 is certainly a good candidate for pHi regulation during mouse embryonic development.

These results could also explain why lactate is necessary for mouse embryos in culture media (Brown and Whittingham, 1991), but not for those used to culture human and bovine embryos. In mice, intracellular lactate allows MCT4 function and thus an optimal embryonic pHi regulation. It has to be understood that MCT can be used to release lactate in case of acidosis (MCT1–4) and pick it up in order to avoid excessive increase in pH (MCT1–2). All these observations raise doubts about the need for the high concentrations of lactate that are added to mammalian embryo culture media because of the high amounts produced by cumulus cells. Excessive generalization between species induces misleading observations. Again, the mouse embryo seems more equipped to regulate its pH and, in some way, the problem of mitochondrial dispersion after culture *in vitro* can be related to misregulation of pH (Stacpole, 1997).

Free amino acids

The results obtained by Sellens *et al.* (1981) are relevant to the use of these compounds. Mouse embryos have difficulty in balancing their internal stores *in vitro*. They are the only species of embryo where it is preferable to have a decline in amino acids in the endogenous pool rather than a desequilibrium. They are also the only model in which high numbers of blastocysts can be attained in high yields without amino acids in culture media. Nevertheless, the internal amino acid and protein content is always lower *in vitro* than *in vivo*. The inner pool is regulated by the outside concentrations of amino acids, competition between different amino acids for the same transporter (different affinities), degradation/turnover of the proteins, anabolism/synthesis of new proteins, and release/export of the different amino acids.

Competition between different amino acids for the same transporter has probably led to misinterpretations of the concept of 'essential amino acids', especially for the in-vivo situation (Van Winkle, 2001). In fact, the concentration of amino acids is one matter and the ratio of different aminoacids quite another. We have observed (Ménézo *et al.*, 1989; Khatchadourian *et al.*, 1994) that methionine, an 'essential amino acid', is actively transported into the embryo and competes actively with the entry of glycine, a 'non-essential' acid with an important role in the embryo. *In vivo*, i.e. in oviduct and uterus, the concentration of glycine may be 50

Table 3. Methionine uptake (fmol/embryo/h) and S-adenosyl methionine (SAM) formation in mouse and human before genomic activation (from Ménézo *et al.*, 1989).

	Methionine uptake	*SAM + SAH conversion (%)*
Mouse 1-cell	190–210	6.5–7.2
Mouse 2-cell	240–290	8.8
Human 4-cell	770	3.4

Table 4. Effect of SAM inhibitors on mouse early embryo development (starting from 1-cell stage, C57 Black; from Ménézo *et al.*, 1989).

Ethionine (µmol)	0	25	100	500
Blastocyst formation	43/50	15/57	0/33	0/34
Percentage	86	26	0	0
Homocysteine (µmol)	0	25	100	500
Blastocyst formation	39/49	33/49	32/56	0/54
Percentage	79	67	57	0

times that of methionine. Only a high glycine/methionine ratio allows the entry of glycine.

Synthesis of S-adenosyl methionine (SAM)

Methionine one of the so-called category of 'essential amino acids' is the precursor of SAM. S-adenosyl methionine is necessary for the activity of DNA methyl transferase activity (**Table 3**). The de-novo methylation of DNA is essential for early mammalian development (Okano *et al.*, 2000) and for the critical process of gene imprinting. Inhibitors of this process, i.e. homocysteine, ethionine and AZT, can interfere severely with embryo development (**Table 4**: Ménézo *et al.*, 1989). Methionyl tRNA initiates all protein synthesis during mRNA translation. It is obvious that in terms of amino acid metabolism, the mouse is not a good model for humans as compared with the cow. When amino acids are all at the same concentration, competition for transporters inhibits the entry of some amino acids, leading to another form of disequilibrium in the endogenous pool and the so-called 'inhibitory effect'. The total number of amino acid transporters and the relative affinity of each amino acid for these transporters imply a strong regulation.

Co-culture and interactions with embryo metabolism

The co-culture technique differs completely from classical embryo culture technologies. The choice of medium for embryo co-culture studies is critical: it must meet the needs of both the embryo and the feeder cells, which is obviously not the case for all culture media. Similarly, the manner in which the cells are cultured, i.e. with monolayers, cell suspensions, etc., can interfere with the outcome of co-culture. After a critical point corresponding to zygotic gene activation, organ culture/co-culture allows the embryo to achieve a dry matter weight, directly related to cell number, similar to the in-vivo situation (Turner *et al.*, 1994).

Interaction with embryo metabolic pathways is an important aspect of feeder cell action. Possibilities include deficits in enzyme and messenger RNA, inhibition of pathways (e.g. phosphofructokinase: Barbehenn *et al.*, 1974), and enzyme disequilibration (e.g. glucose phosphate isomerase: Ménézo and Khatchadourian, 1990). These factors can result in an inability to metabolize some compounds, waste of energy and accumulation of superfluous products. For example, in the case of glucose, high external concentrations in conventional culture media lead *in vitro* to the accumulation of glycogen in mouse embryos. This does not occur *in vivo* and/or in co-culture (Khurana and Wales, 1987). Co-culture also provides a favourable RedOx potential, i.e. equilibrium between reducing and oxidative substances in favour of a reducing value, which is rarely taken into account (Ouhibi *et al.*, 1990).

Amino acids can be toxic through their catabolism and the formation of ammonium ions (Gardner and Lane, 1993). In open in-vitro culture conditions, ammonia forms ammonium carbonate and/or bicarbonate, both highly unstable, especially at alkaline pH. Ammonia is eliminated or recycled enzymatically *in vivo* (Ménézo *et al.*, 1993; Lane and Gardner, 1995), or in co-culture systems, through formation of alanine, glutamine and asparagine.

Co-culture experiments have been carried out in mice, where Vero cells (B2/TCM 199) do not sustain early preimplantation development (Ouhibi *et al.*, 1990). They have been extensively and successfully used with human embryos, and are still used on a large scale with bovine embryos (Menck *et al.*, 1997), and especially for cloned embryos (Le Bourhis *et al.*, 1998).

Growth factors

This situation is very controversial. First of all, a decision must be made to consider mRNAs or proteins. Then, it must be decided whether growth factors operate through an autocrine or a paracrine (maternal) mechanism. Positive effects of embryo culture in groups has been linked in mouse to growth factors (Paria and Dey, 1990). The embryo might be able to synthesize its own and release some of them into medium, so neighbouring embryos could be able to incorporate and utilize them. This would mean that a lone embryo is not able to use its own growth factors in an internal 'autostimulation' loop.

Positive effects on groups of embryos cultured in the same drop are generally denied for humans and questionable for cows. Feeder cells (especially Vero cells) release many growth factors. However, the use of sequential media has led to the conclusion that they play a marginal effect. In the cow, they could be involved in the large offspring syndrome, when serum is added to culture. As far as we know (Ménézo *et al.*, 1999), this syndrome does not exist in humans. Insulin is usually added for the second phase of culture: whether or not it has the same effect as IGF1 is questionable (Lighten *et al.*, 1998).

Growth factors may act through a balance between stimulatory and inhibitory effects. It is important to note that growth hormone and its receptor are present in mouse (Pantaleon *et al.*, 2000) and bovine embryos (Izadyar *et al.*, 2000). There is not enough global information to encourage the addition of growth factors to sequential media (Teruel *et al.*, 2000). No

single good model exists to enable assessments of the impact of growth factors on early human embryogenesis.

Intrinsic factors in the regulation of embryo development

Paternal, maternal and cytogenetic factors could interfere with preimplantation development. Chromosomal abnormalities are one such factor, a most acute problem for human embryos (Benkhalifa *et al.*, 1996; Gras *et al.*, 1999). It is also a well-known problem in bovine embryo transfer biotechnology (Viuff *et al.*, 2000), but to a lesser extent. In-vitro culture technology is probably one key to this problem. Equal attention must be devoted to the impact of oxygen tension.

Maternally-inherited products from the oocyte, timing of genomic activation, and temporal pattern of gene expression during initial development of the human embryo also have their own impact. Paternal factors share the same targets in bovine and human embryos (Janny and Ménézo, 1994; Navarra *et al.*, 1996; Comizzoli *et al.*, 2000).

Embryo freezing

It is common knowledge that an extended in-vitro culture period makes the embryo more sensitive to freezing and thawing injury. Most of the animal embryos collected *in vivo* are frozen at the blastocyst stage. Human IVF embryos are cryopreserved during pronucleate, cleavage and blastocyst stages, using propanediol or dimethyl sulphoxide (DMSO). We designed our human IVF blastocyst freezing protocols using knowledge and skills gained with the control of bovine IVM/IVF blastocysts. This model was, in our hands, the best achievable (Ménézo and Veiga, 1997). Freezing mouse early stage embryos and blastocysts is rather too easy to be a really discriminant model (Van den Abbeel and Van Steirteghem, 1987).

Corpus luteum and implantation

The problem of embryo implantation looms very large in human IVF. Our studies on the incidence of blastocyst implantation in pregnant and other treated patients reveal a huge discrepancy related to uterine receptivity. The overall implantation rate per embryo is, according to the transfer stage, between 10 and 30% for blastocysts. In pregnant patients, it is generally >50%. This implies that for the same population of embryos, the quality of the endometrium determines the chances of implantation.

Leukaemia inhibitory factor (LIF) is mandatory for implantation in mice, which is not generally the case for humans. Both mouse and human fetuses have haemochorial placentation as compared with an epitheliochorial type in cows. Obviously, the nature of implantation in the mouse is closer to humans than to cows.

Prolactin must be released at the time of coitus in mice to induce the transformation from cyclic to pregnant corpus luteum. There are no similar demands either in humans (see frozen–thawed embryo transfers) or in cows, where a cyclic corpus luteum fits well for embryo transfer. Embryonic signalling is necessary for the maintenance of pregnancy in humans (human chorionic gonadotrophin) as well as in cow (interferon Tau). The synthesis of enkephalins from pro-opiomelanocortin has been demonstrated in corpora lutea of humans, mice and cows without defining a clear role for these neuromediators (Cupo *et al.*, 1987).

Conclusion

To conclude, it is difficult to determine which is the best animal model for human IVF. There is no unique scheme and comparisons depend on the target of the study in question. To train embryologists, and to assess the pharmacological aspects of testing materials and media for IVF, 1-cell embryos, but certainly not 2-cell mouse embryos, offer an interesting model. In term of cryobiology, major problems are only detected with mouse embryos. In all topics under investigation, the available genetic strains play a major role in helping overcome many of the basic problems. Data from mice do not really fit into work on improvements in IVF methodology that take us constantly to the limits of research. The bovine model is more appropriate. One of the most striking problems is that it is uncertain whether basic data collected from mice (see earlier sections on the onset of transcription, modification of chromatin structure, and nucleocytoplasmic relationships) will fit in well with other mammalian embryos. In the near future, however, embryo stem cell technology gathered via the mouse model and cloning technology gathered from bovine IVM/IVF technology will help if 'therapeutic cloning' is proposed and accepted for human beings.

References

Ao A, Erickson RP, Winston RML *et al.* 1994 Transcription of paternal Y-linked genes in the human zygote as early as the pronucleate stage. *Zygote*, **2**, 281–289.

Aoki F, Worrad DM, Schultz RM 1997 Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Developmental Biology*, **181**, 296–307.

Barbehenn EK, Wales RG, Lowry OH 1974 The explanation for the blockade of glycolysis in early mouse embryo. *Proceedings of the National Academy of Sciences of the USA*, **71**, 1056–1060.

Benkhalifa M, Ménézo Y, Janny L *et al.* 1996 Cytogenetics of uncleaved oocytes and arrested zygotes in IVF programs. *Journal of Assisted Reproduction and Genetics*, **13**, 140–148.

Betteridge KJ, Rieger D 1993 Embryo transfer and related techniques in domestic animals, and their implications for human medicine. *Human Reproduction*, **8**, 147–167.

Brown J, Whittingham D 1991 The roles of pyruvate, lactate and glucose during preimplantation development of embryos from F1 hybrid mice *in vitro*. *Development*, **112**, 99–105.

Chi MM, Manchester JK, Yang VC *et al.* 1988 Contrast in levels of metabolic enzymes in human and mouse ova. *Biology of Reproduction*, **39**, 295–307.

Comizzoli P, Marquant-Le Guienne B, Heyman Y *et al.* 2000 Onset of the first S-phase is determined by a paternal effect during the G1-phase in bovine zygotes. *Biology of Reproduction*, **62**, 1677–1684.

Cortvrindt R, Smitz J 2001 *In vitro* follicle growth: achievements in mammalian species. *Reproduction in Domestic Animals*, **36**, 3–9.

Cupo A, Ménézo Y, Bueno L 1987 Enkephalin production by the corpus luteum *Neuropeptides*, **9**, 237–245.

Dale B, Ménézo Y, Cohen J *et al.* 1998 Intracellular pH regulation in the human oocyte. *Human Reproduction*, **13**, 964–970.

Dimmer KS, Friedrich B, Lang F *et al.* 2000 The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. *Biochemical Journal*, **35**, 219–227.

El Mouatassim S, Guérin P, Ménézo YJR 1999 Expression of genes encoding antioxidant enzymes in human and mouse oocytes during final stages of maturation. *Molecular Human Reproduction*, **5**, 720–725.

Gardner DK, Lane M 1993 Amino acods and ammonium regulate mouse embryo development in culture. *Biology of Reproduction* **48**, 377–385.

Gibb CA, Poronok P, Day ML *et al.* 1997 Control of cytosolic pH in 2-cell embryos: role of H^+/lactate cotransport and Na^+/H^+ exchange. *American Journal of Physiology*, **273**, C 404–419.

Gras L, Gianaroli L, Magli C *et al.* 1999 High rates of aneuploidy in human embryos that fail to grow to blastocysts *in vitro*. Abstracts of the 11th World Congress on *In vitro* Fertilization and Human Reproductive Genetics, Sydney, May, S-012.

Hérubel F, El Mouatassim S, Frydman R, Ménézo Y 2002 Genetic expression of MCT isoforms 1, 2, 4 and basigin during human preimplantation development. *Zygote* (in press).

Ho Y, Doherty AS, Schultz RM 1994 Mouse preimplantation embryo embryo development *in vitro*: effect of sodium concentration in culture media on RNA synthesis and accumulation and gene expression. *Molecular Reproduction and Development*, **38**, 131–141.

Izadyar F, Van Tol HTA, Hage WG *et al.* 2000 Preimplantation bovine embryos express mRNA of growth hormone receptor and respond to growth hormone addition during *in vitro* development. *Molecular Reproduction and Development*, **57**, 247–252.

Janny L, Ménézo YJR 1994 Evidence for a strong paternal effect on human preimplantation embryo development and blastocyst formation. *Molecular Reproduction and Development*, **38**, 36–42.

Khatchadourian C, Guillaud J, Menezo YJR 1994 Interactions in glycine and methionine uptake, conversion and incorporation into proteins in the preimplantation mouse embryo. *Zygote*, **2**, 301–306.

Khurana NK, Wales RG 1987 Effects of coculture with uterine epithelial cells on the metabolism of glucose by mouse morula and early blastocyst. *Australian Journal of Biological Science*, **40**, 389–395.

Lane M, Gardner DK 1995 Removal of embryotoxic ammonium from the culture medium by in-situ enzymatic conversion to glutamate. *Journal of Experimental Zoology*, **271**, 356–363.

LeBourhis D, Chesne P, Nibart M *et al.* 1998 Nuclear transfer from sexed parent embryos in cattle: efficiency and birth of offspring. *Journal of Reproduction and Fertility*, **113**, 343–348.

Lighten AD, Moore GE, Winston RM *et al.* 1998 Routine addition of human insulin growth factor-I ligand could benefit clinical *in vitro* fertilization culture. *Human Reproduction*, 1998, **13**, 3144–3150.

MacGrath J, Solter D 1984 Completion of mouse embryogenesis requires both the maternal and the paternal genome. *Cell*, **37**, 179–183.

Memili E, First NL 1999 Control of gene expression at the onset of bovine embryonic development. *Biology of Reproduction*, **61**, 1198–207.

Menck C, Guyader-Joly C, Peynot N *et al.*1997 Beneficial effect of Vero cells for developing IVF bovine eggs in two different coculture systems *Reproduction*, *Nutrition*, *Development* **37**, 141–150.

Ménézo Y, Khatchadourian C 1990 Involvement of glucose 6 phosphate isomerase activity (E.C. 5.3.1.9) in the mouse 2-cell block *in vitro*. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences*, *Paris, Serie III*, **310**, 297–301.

Ménézo Y, Veiga A 1997 Cryopreservation of blastocyst. *In vitro* fertilization and assisted reproduction. Proceedings of the Xth World Congress on *in vitro* fertilization and assisted reproduction, Vancouver, pp. 49–53.

Ménézo Y, Khatchadourian C, Gharib A *et al.* 1989 Regulation of S-adenosyl methionine synthesis in the mouse embryo. *Life Sciences*, **44**, 1601–1609.

Ménézo Y, Janny L, Khatchadourian C 1993 Embryo quality and co-culture. In Mastroianni L, Coelingh Bennink HJT, Suzuki S, Verner HM (eds) *Gamete and Embryo Quality*. Proceedings of the Fourth Organon, Round Table Conference, Thessaloniki, Greece, pp. 139–155.

Ménézo Y, Chouteau J, Torello MJ *et al.* 1999 Infant birth weight and sex ratio after human blastocyst stage transfer. *Fertility and Sterilility*, **72**, 221–223.

Mikkelsen AL, Smith S, Lindenberg S 2000 Possible factors affecting the development of oocytes in in-vitro maturation. *Human Reproduction*, **5**, 11–17.

Muggleton-Harris A, Whittingham DG, Wilson L 1982 Cytoplasmic control of preimplantation development *in vitro* in the mouse. *Nature*, **299**, 460–462.

Navara CS, First NL, Schatten G 1996 Phenotypic variations among paternal centrosomes expressed within the zygote as disparate microtubule lengths and sperm aster organization: correlations between centrosome activity and developmental success. *Proceedings of the National Academy of Sciences of the USA*, **93**, 5384–5388.

Okano M, Bell DW, Haber DA *et al.* 2000 DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, **90**, 247–257.

Ouhibi N, Hamidi J, Guillaud J *et al.* 1990 Co-culture of 1-cell mouse embryos on different cell supports. *Human Reproduction*, **5**, 737–743.

Pampfer S 2000 Apoptosis in rodent peri-implantation embryos: differential susceptibility of inner cell mass and trophectoderm cell lineages—a review. *Placenta*, **14**, S3–S10.

Pantaleon M, Whiteside EJ, Harvey MB *et al.* 2000 Functional growth hormone (GH) receptor and GH are expressed by preimplantation mouse embryo: a role for GH in early embryogenesis. *Proceedings of the National Academy of Sciences of the USA*, **94**, 5125–5130.

Paria BC, Dey SK 1990 Preimplantation embryo development *in vitro*: cooperative interactions among embryos and role of growth factors. *Proceedings of the National Academy of Sciences of the USA*, **87**, 4756–4760.

Sellens MH, Stein S, Sherman MI 1981 Protein and free amino acid content in preimplantation mouse embryos and in blastocysts under various culture conditions. *Journal of Reproduction and Fertility*, **61**, 307–315.

Stacpoole PW 1997 Lactic acidosis and other mitochondrial disorders. *Metabolism*, **46**, 306–321.

Teruel M, Smith R, Catalano R 2000 Growth factors and embryo development *BioCell*, **24**, 107–122.

Trounson A, Anderiesz C, Jones G 2001 Maturation of human oocytes *in vitro* and their developmental competence. *Reproduction*, **121**, 51–75.

Turner K, Rogers AW, Lenton EA 1994 Effect of culture *in vitro* and organ culture on the dry mass of preimplantation mouse embryos. *Reproduction, Fertility, Development*, **6**, 229–234.

Van den Abbeel E, Van Steirteghem A 1987 Cryopreservation of *in vitro* cultured mouse preimplantation embryos. *Annales de Biologie Clinique (Paris)*, **45**, 460–463.

Van Winkle LJ 2001 Amino acid transport regulation and early embryo development. *Biology of Reproduction*, **64**, 1–12.

Viuff D, Greve T, Avery B *et al.* 2000 Chromosome aberrations in *in vitro*-produced bovine embryos at days 2–5 post-insemination. *Biology of Reproduction*, **63**, 1143–1148.