Abnormally fertilised embryos in ivf

Normal fertilisation is characterised by the visualisation of two distinct pronuclei (2PN) 16-20 hours after conventional insemination or intracytoplasmic sperm injection. Abnormalities of fertilisation are also observed with either 1 pronucleus (1PN), ≥3 pronuclei or cleavage without observation of nuclei (0PN). Oocytes demonstrating ≥ 3PN are not transferred as they are thought to be triploid (Feenan and Herbert, 2006). However, difficulty arises when there are no normally fertilised (2PN) embryos available for transfer, as to whether 1PNs or 0PNs that cleave should be considered for transfer.

Monopronuclear embryos

Monopronuclear (1PN) embryos are seen in varying quantities from 1. 6% – 7. 7% (Dasig et al. 2004) with conventional IVF, compared to a higher percentage (4. 9-11. 4%) in ICSI cases (reviewed by Feenan and Herbert, 2006). In most cases, these 1PN embryos can be disregarded in favour of normally fertilised embryos. However, where there are only monopronuclear embryos available for transfer, the question arises as to whether it is safe to consider these embryos for transfer.

The mechanism for the appearance of 1PN is thought to be due to parthenogenic activation, asynchrony in the appearance or arrest in formation of pronuclei or male and female pronuclear fusion (Liao et al. 2009).

Parthenogenic activation of oocytes occurs when there is an internal or external trigger for the resumption of meiosis in the oocyte, independent of the presence of sperm. A single haploid pronucleus is observed, despite the

embryo not being fertilised. These parthenotes are capable of cellular division to the 8-cell stage but fail to progress to the blastocyst stage due to the absence of the paternal genome (Levron et al. 1995).

Pronuclei are normally observed 16-20 hours after insemination or injection. However, Nagy et al.(1998) report a greater proportion of IVF oocytes (45. 7%) developing pronuclei asynchronously compared to ICSI oocytes (17. 6%) but in general, the second pronucleus should be visible 30 minutes after the first pronucleus (Payne et al. 1997). For this reason, 1PN embryos are generally rechecked 4 hours after the original fertilisation check to see if the second pronucleus can be visualised. If the second pronucleus is visualised it can be potentially be considered normally fertilised as long as it shows normally embryo development.

Fusion of the male and female pronuclear has also been suggested as a mechanism of the formation of diploid 1PN embryos. It has been described (Levron et al. 1995) that the nuclear membranes do not actually fuse but the unipronucleate zygotes are formed by the enclosure of the juxtaposed pronuclei in a common pronuclear envelope. In these cases, a larger pronucleus is seen compared to the size of normal pronuclei and they contain more nucleioli. The extrusion of two polar bodies also indicates that chromosomes have not been retained.

A further cause for the observation of a single pronucleus is the possible arrest of the second pronucleus, which results in the male or the female chromatids failing to form a pronucleus. Flaherty et al (1995) proposes that the failure of the paternal pronucleus to form in ICSI cases is attributable to

either ejection of sperm (20%), retention of the condensed sperm head (28%) or partial decondensation of the sperm head (52%). Failure in the formation of maternal pronuclei could be attributable to the chromosomes continually condensing, forming a compact nucleus or the failure of meiotic resumption and would result in an oocyte demonstrating 1PN and 1PB.

Feenan and Herbert (2006) showed that a greater proportion of IVF (56. 9%) 1PN zygotes are diploid compared to only 28. 0% in ICSI. Therefore, it is suggested that 1PN embryos arising from ICSI should not be considered for transfer. However, 1PN embryos arising from conventional IVF where a single large pronucleus and two polar bodies are visualised can be considered as long as normal embryonic development is observed and there are no other normally fertilised embryos available for transfer. Embryos where a single ‘ normal’ sized pronucleus is observed, irrespective of the number of polar bodies, should not be considered as they are more likely be due to the parthenogenic activation or failure of paternal or maternal pronuclear formation.

Tripronuclear embryos

Tripronuclear embryos can be dispermic (caused by two sperm entering the oocyte) or digynic (where the extra pronucleus is of maternal origin). Dispermy is the most common fertilisation anomaly and is commonly observed as three pronuclei and two polar bodies. Digynic embryos often arise after intracytoplasmic sperm injection (ICSI) (5. 1-7. 4% described by Feenan and Herbert, 2006), and are thought to be a consequence of sperm injection near the meiotic spindle of the oocyte, possibly causing disruption of the correct chromosome segregation (Macas et al., 1996). The number of

3PN embryos observed in ICSI cases are generally less compared to conventional IVF, due to monospermic injections. Feenan and Herbert (2006) conclude that the majority of 3PN embryos have triploid chromosome complements (61. 8%), mosaic arrangements (25. 2%) and only 12. 6% of embryos had a diploid chromosome complement and therefore should never be considered for transfer.

Enucleation of multipronuclear zygotes has been described (Ivakhnenko et al. 2000). In this technique, the extra pronucleus is microsurgically removed in the attempt to leave a diploid embryo. The major limitation of this procedure is the difficulty in identifying which is the ‘ extra’ pronucleus. It is possible that if the wrong pronucleus is removed, this may lead to the embryo inheriting two sets of maternal or paternal chromosomes.

There is evidence of naturally occurring triploid pregnancies but virtually all of these, result in miscarriage (Feng and Hershlag, 2003) and also neonatal deaths. Feenan and Herbert (2006) summarise the foetal abnormalities arising from triploid embryos which include major central nervous system defects, abdominal wall defects and intrauterine growth retardation.

As a high proportion of tripronuclear embryos are triploid or mosaic 3PN embryos coupled with the extremely poor outcome of triploid pregnancies, it is advisable that these embryos are not considered for transfer.

Apronuclear (0PN) embryos

Munne and Cohen (2008) describe that 1% of zygotes with two polar bodies do not show pronuclei but can go on to show normal cleavage. Preliminary studies by Manor et al. (1996) showed that 57% of these zygotes were

diploid, 30% polyploidy or mosaic and 13% aneuploid. The suggested mechanism for the failure of observation of the pronuclei was thought to be the accelerated dismantling of the pronuclear membranes and attributable to ‘ intriguing biological variation’ rather than abnormal cleavage. There is very limited evidence for the use of these zygotes in treatment, however, Manor et al. (1996) did describe an ongoing pregnancy with a karyotype of 46XX from a zygote that appeared to be unfertilised. Noyes et al. (2008) reported that none of the 0PN embryos that were analysed reached blastocyst stage on day 5, however 3% had euploid karyotypes after biopsy, this led to the suggestion that 0PN embryos should not be considered for transfer. There is only limited data available on the chromosomal status of 0PNs and therefore it is difficult to ascertain their reproductive potential, but at present it is safer not to consider these embryos for transfer.

Discussion

The available evidence, although limited, suggests that in the event of the absence of ‘ normally’ fertilised embryos, the majority of ‘ abnormally’ fertilised embryos should not be considered for transfer. Triploidy rates in embryos displaying 3PN are generally high and in vivo pregnancies of triploid embryos have an extremely poor prognosis and so should not be transferred. Monopronuclear embryos where the single pronucleus is of regular size, irrespective of the number of polar bodies, should not be considered for transfer due to the high likelihood that the embryos do not have the adequate chromosome complement. Monopronuclear embryos displaying a single, large pronucleus and two polar bodies can however be considered for transfer in the event of no normally fertilised (2PN) embryos. Fusion of male

and female pronuclear into a single membrane envelope, thus creating a large pronuclei, is thought to be the mechanism for these observation of the 1PN and are thought to contain the correct chromosome complement. 1PN, as a result of ICSI, should not be transferred due to the reduced likelihood of these embryos being diploid. Little evidence is also available for the consideration of 0PN embryos for transfer, but where two polar bodies are observed and normal cleavage rates are observed, they can be considered for transfer as it is likely that there has been atypical, accelerated dismantling of pronuclear membranes prior to fertilisation checks.

In all cases, the embryo should be evaluated at least until day 3 of development and only considered for transfer if ‘ normal’ patterns of development are observed. Adequate patient counselling must be given prior to the transfer of any of these embryos where ‘ normal’ fertilisation has not been observed but the embryos have proceeded to cleavage stage. They must be fully aware of the risk of implantation failure or miscarriage before considering whether to proceed with the embryo transfer.

Conclusion

In most IVF/ICSI treatment cycles, adequate numbers of normally fertilised embryos are obtained and ‘ abnormally’ or failed to fertilise embryos can be disregarded in preference for the 2PN embryos. In rare cases where no normal fertilisation has been observed, it is thought that zygotes with a single, large pronucleus and two polar bodies, as a result of conventional insemination can be considered for transfer as well as 0PN embryos also displaying two polar bodies, as long as normal embryonic development is observed. All embryos resulting from ≥3PN and 1PN zygotes where the

pronucleus is of a normal size or abnormal fertilisation as a result of ICSI should not be considered for transfer due to the increased risk of abnormality.