Reducing the Multiple Pregnancy Rate in ART

Mark Bowman MBBS, PhD, FRANZCOG, CREI*

** Visiting Medical Officer Royal Price Alfred Hospital*

The greatest risk element in any form of assisted conception, is the risk to a fetus within a multiple pregnancy, even with twin implantation. Recent research has examined the contribution of single embryo transfer in reducing the incidence of multiple gestation within IVF. Two recent studies from our assisted conception centres have demonstrated that single embryo transfer can be undertaken without compromising the patient's ultimate chance of having a successful ongoing pregnancy. The first study was a randomized trial comparing the pregnancy rates in women having two embryos transferred at day two or three after oocyte collection, with that of a second group undergoing single blastocyst transfer. The results revealed an equivalent ongoing pregnancy rate but a significant reduction in the twin rate (to zero) in the single transfer group. A second study examined the cumulative pregnancy rate in women with two or more suitable blastocysts after oocyte collection for transfer, when the final chance of pregnancy was calculated after all embryos had been transferred fresh and in subsequent frozen-thaw embryo transfers. The results were calculated for two groups of women in this data set, one receiving a single embryo for transfer fresh and the second group having two embryos transferred fresh. There was an initial improvement in the fresh embryo transfer group having two transferred (59% vs 44% for the single transfer group), but when additional pregnancies from frozen-thawed pregnancies were added, there was no difference in the final cumulative pregnancy rate in either group (74% for the single transfer group and 70% for the double transfer group). In contrast, 28% of overall pregnancies in the (initial) double transfer group were multiple, whereas only 5% of the (initial) single transfer group were twins. The development of high quality incubation systems within IVF that allow for both blastocyst culture and successful cryopreservation allow for both a high pregnancy rate and the development of strategies to reduce the multiple pregnancy rate.

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Many of our infertile patients actively desire twins and even higher order multiple pregnancy, perceiving this to be a positive outcome from infertility treatment. Most will form the view that relative to the effort and cost involved with assisted conception, twins are a favourable outcome and might lead to a reduced need for further management as their family will be completed with only one course of treatment. Often, their treating practitioner overtly or covertly tolerates this view. Nonetheless, multiple pregnancy remains one of the greatest concerns within assisted conception treatment and active measures should be undertaken to reduce the incidence of even twinning within such treatment.

The paper will review the likelihood of multiple pregnancy within assisted conception and the problems that can emerge as a result. Data will be presented to argue that single embryo transfer, preferably at the day five (blastocyst) stage, is currently the most useful tool to achieve the highest likelihood of successful ongoing pregnancy, whilst reducing the multiple pregnancy rate to a similar level to that experienced following spontaneous conception.

Correspondence to : Bowman M, Clinical Director, Sydney IVF, Visiting Medical Officer Royal Price Alfred Hospital, Australia.

Morbidity and Mortality associated with twins

There are significant increases in perinatal morbidity associated with multiple pregnancy. Multiple pregnancies are more susceptible to intrauterine growth retardation with a triplet's birth weight only half that of a singleton, on average. Follow up studies of growth-retarded twins demonstrate a persistence of short stature and lower weight percentiles[1].

Low birth weight infants (< 2500 grams) are

three times as likely to suffer physical and mental handicaps[2]. Many babies from multiple pregnancies require extended treatment in neonatal intensive care units at a cost of thousands of dollars per day and overall, 78% of triplets require admission compared with only 15% of singletons.

Twin pregnancies are also more susceptible to abortion and the rate of major malformations is double that of a singleton, at about 2%[3].

The National Perinatal Statistics Unit of Australia collects data on the outcomes of pregnancies in that country. The perinatal mortality data for twins and higher order multiple pregnancies, irrespective of whether conceived naturally or through assisted conception, show some disturbing trends.

In 2000, multiple pregnancy accounted for 1.6% of all births in Australia. Twins accounted for 15.1 per 1000 confinements in that year and 100 of just over 4000 multiple pregnancy confinements were triplet gestations. The perinatal death rate for singletons (defined as a death from between 20 weeks gestation and one month post delivery) was 7.7 per 1000 births, but 29.5 per 1000 births for twins. For other multiple births, a perinatal mortality rate of 53.0 per 1000 births was recorded[4].

Within assisted conception, poorer outcomes are noted. Thirty four percent of assisted conception births in Australia and New Zealand in 2000 were twin pregnancies and 2.4% triplets. The perinatal death rate for twins in that year from assisted conception was 34.3 per 100 births and 62.0 per 1000 births for triplets[5].

Multiple pregnancy rates following clomiphene citrate and controlled ovarian stimulation with insemination (COH-IUI)

There is a paradox in that so called "low level" assisted conception techniques, whilst being considered to be more natural in their approach, actually have a moderate rate of multiple pregnancy because of the uncontrolled number of mature oocytes that may develop. The unmonitored use of clomiphene citrate is an example of this problem, as is the use of controlled ovarian stimulation with gonadotrophins in combination with intrauterine insemination (COH-IUI).

Such is the concern that unmonitored clomiphene citrate usage has in leading to multiple pregnancy (in excess of 10% of all clomiphene pregnancies being multiple) that the Royal College of Obstetricians and Gynaecologists (RCOG) guidelines recommend ultrasound monitoring for all patients commencing this agent[6].

The use of COH-IUI, particularly in the United States, has led to concerns as to the unwarranted rate of multiple pregnancy that results from this form of management. Gleicher et al reasoned that the only effective way to reduce multiple pregnancy rates from COH-IUI (where twinning often accounts for at least 20% of pregnancies) was to reduce the dose of FSH stimulation used[7], but that in doing so the resultant pregnancy rate fell to levels that made this treatment not cost effective, relative to invitro fertilization (IVF)[8].

Healy et al reported their results from using low dose FSH stimulation and IUI and noted one triplet pregnancy in their cohort (2% of pregnancies reported) and a 10% incidence of twins[9]. However the overall pregnancy rate per cycle was only in the order of 10%. In contrast, IVF pregnancy rates are significantly higher when treatment is conducted appropriately[10].

Whilst therefore it would appear that twin pregnancy can be reduced using COH-IUI, even in the most conservative treatment programs the risk of twinning is still at least five times the natural incidence.

The use of single embryo transfer in invitro fertilization (IVF)

A number of recent studies have examined the use of single embryo transfer within IVF, mostly utilising embryo transfer at day three[11,12]. The pregnancy rates in these studies revealed a slight decrease in the chance of initial pregnancy, but with the effective elimination of the risk of twins.

Correspondingly, the extended culture of embryos to the blastocyst stage (five days after oocyte collection) has been reported to improve implantation rates, through better embryo selection[13], although incubation and culture media conditions are critical for success.

In Sydney, as our assisted conception pregnancy rates have improved (through both improved incubation conditions and the culturing of embryos to the blastocyst stage), there has been a corresponding increase in multiple pregnancy rates. At Sydney IVF, in our sites at our private hospital facility and also at Royal Prince Alfred Hospital, twin rates of nearly 40% per pregnancy, where two embryos had been transferred within IVF, were noted. As a result, a randomised controlled trial was undertaken, comparing the pregnancy rates and multiple pregnancy rates after single and double embryo transfer[14].

Our aim was to compare the pregnancy and multiple pregnancy rate, between the transfer of two

embryos three days after oocyte collection (ie at the six to eight cell embryo stage) and the outcomes following the transfer of a single blastocyst embryo five days after oocyte collection.

Fifty nine patients under 38 years of age were prospectively randomised to receive either two embryos at day three or a single embryo at day five. In the double embryo transfer group the clinical pregnancy rate per embryo transfer was 41.4% (12/29) of which seven (58.3%) were twin pregnancies and five (41.7%) were singleton. In the single embryo transfer group the clinical pregnancy rate per embryo transfer was 46.7% (14/30) and all were singleton (Table 1). The overall twin pregnancy rate in the two embryo transfer group was found to be statistically different to the single transfer group, P = 0.005.

In addition, for those women randomized to single embryo transfer at day five, the embryologist made a choice as to which were the most suitable embryos to transfer at day three, those embryos were then observed to day five. In nine of 24 such observations, the embryo that were deemed to be the "best" embryos for transfer on day three did not develop into appropriate blastocysts by day five.

As a result of this study, our routine protocol at Royal Prince Alfred Hospital is to transfer only one embryo at day five, in women under 38 years of age undertaking their first or second stimulation.

Recently, Gardner et al have also examined the return from single blastocyst transfer. They randomized 48 women undergoing IVF with at least 10 suitable follicles, to receive either one or two blastocysts for embryo transfer. There was a modest difference in the pregnancy rate but a significant difference in the rate of twins (0% in the single transfer group and 47.4% twin rate in the two embryo transfer group)[15].

Thus it would appear that the successful culturing of embryos to the blastocyst stage with single embryo transfer is a useful technique in reducing the multiple pregnancy rate within IVF, without compromising the fresh embryo transfer rate, relative to the transfer of two embryos.

Table 1. Comparison of outcomes where two embryos were transferred at cleavage stage, vs single blastocyst transfer[14]

	Day 3 embryos transfer	Day 5 Single embryo transfer
# ET	29	30
# clinical preg	12 (41.1%)	14 (46.7%)
# twin pregs	7 (58.3%)*	0 (0%)*

P = 0.005

Cumulative outcomes per oocyte collection, utilising blastocyst culture

One further mechanism to reduce multiple pregnancy rate is to maximize the return from frozen-thawed embryo transfer[16]. Following the collection of oocytes and the creation of embryos, the elective transfer of single embryos can be undertaken for the initial fresh cycle and for subsequent frozen embryo transfers. Using this strategy, patients and their doctor need to weigh the short term cost and time disadvantage of this approach, against avoiding the longer term potential consequences of multiple pregnancy.

Sydney IVF has compared the cumulative outcomes per oocyte collection in women undertaking an elective single embryo transfer policy, where two or more suitable blastocyst embryos where available for fresh transfer, with those electing to have two embryos transferred[17]. Between 1998 and 2001, patients aged less than 38 years with at least two good quality blastocyst embryos were invited to have one embryo transferred fresh with residual embryos cryopreserved. All patients had long down regulation and ovarian stimulation with FSH. Transvaginal oocyte retrieval was undertaken and eggs inseminated with conventional IVF or intracytoplasmic sperm insertion (ICSI) according to sperm parameters. All zygotes were cultured to the blastocyst stage using stage specific Sydney IVF Media.

For patients having single embryo transfer (n = 111), 49 (44%) had an ongoing pregnancy (defined as an intrauterine gestation seen at ultrasound, 4-5 weeks after embryo transfer). Two percent of these pregnancies were (monozygotic) twins. Of patients having two embryos transferred fresh (n = 274), 161 conceived (59%), with 71 twin pregnancies noted (overall twin rate 44% of pregnancies). Table 2 summarizes these data.

For both groups, patients who did conceive following fresh transfer returned for the transfer of frozen-thawed embryos, if available (Table 3). Further ongoing pregnancies were attained in both groups. The final chance of attaining pregnancy in the (initial) single embryo transfer group was 74% and 70% for the (initial) double embryo transfer group. In contrast, the final twin pregnancy rate for the single embryo group was 5%, compared to 28% for the double transfer group.

These data suggest that a policy a blastocyst culture and with successful cryopreservation of residual

Table 2. Pregnancy rates after the fresh transfer of one or two blastocysts, for women less than 38 years of age with two or more suitable embryos[17]

	One transferred	Two transferred
# embryo transfers	111	274
# clinical pregnancies	49 (44%)	161 (59%)
# fetal heartbeats (HB)	50	232
% fetal HB / embryo transferred	45%	42%
# twin pregnancies	1	71
% twins per pregnancy	2%	44%

Table 3. Additional frozen-thaw embryo transfer pregnancies and cumulative outcomes[17]

	One ET (fresh)	Two ET (fresh)
No. frozen transfers	112	105
No. clinical pregnancies (% per ET)	33 (29%)	29 (28%)
Total no. patients pregnant (fresh plus frozen)	83	190
% Patients pregnant per egg collection and ET	74%	70%
Overall twin %	5%	28%

day five embryos can lead to a reduction in multiple pregnancy rates without reducing the younger patient's ultimate chance of pregnancy per oocyte collection.

Conclusion

Multiple pregnancy rates can be reduced in ART, but increasingly more sophisticated treatments may be warranted to achieve this aim. Whilst simpler treatments such as COH-IUI are attractive, appropriate pregnancy rates may only be achievable at the cost of a significant multiple pregnancy rate. In contrast, IVF with extended embryo culture, combined with successful embryo cryopreservation, is emerging as the most appropriate strategy to maximize the chance of attaining pregnancy whilst minimizing the risk of multiple pregnancy.

Patients need to counselled early in the consultative and investigative phase of their infertility as to the potential risks of twin and higher order multiple gestations. It should be noted that if there is no long term lowering of the overall pregnancy rate through judicious single embryo transfer within IVF, then the chance of having a healthy liveborn child is higher following single embryo transfer than it is from double embryo transfer, given the significantly higher perinatal morbidity and mortality from multiple gestations.

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