Effect of profound suppression of luteinizing hormone during treatment with gonadotrophin-releasing hormone analogue and purified follicle stimulating hormone upon development of cryopreserved embryos

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In response to previously published evidence from monkeys, this study examined the influence of the degree of luteinizing hormone (LH) suppression during the follicular phase of the stimulation cycle, upon cryopreserved embryo survival and development. The LH concentration of the mid-follicular phase was assessed in 250 in-vitro fertilization (IVF) cycles treated with gonadotrophin-releasing hormone analogue (GnRHa) and either purified follicle stimulating hormone (FSH) or human menopausal gonadotrophin (HMG), and was related to the performance of cryopreserved embryos in 351 subsequent embryo transfer cycles. Rates of embryo survival, embryo development rates, implantation rates, and pregnancy rates were examined with respect to the LH concentration recorded in the mid-follicular phase. In contrast to experimental evidence from other primates, there was no significant influence of the follicular phase LH concentration upon any of the parameters examined.

Key words: cryopreserved embryos/embryo development/GnRHa/LH/purified FSH

Introduction

The two-cell, two-gonadotrophin model of follicular steroidogenesis postulates that both follicle stimulating hormone (FSH) and luteinizing hormone (LH) are needed for follicular growth and maturation in the human. LH acts on the theca cells to produce androgen substrate, which undergoes aromatization to oestradiol within the granulosa cell compartment under the influence of FSH. Evidence from women with hypogonadotropic hypogonadism undergoing ovulation induction with exogenous gonadotrophins indicates that follicular growth in the human may proceed under the influence of FSH alone with negligible LH activity, but that the oestradiol output demands some LH activity, although neither the amount required, nor the nature of its kinetics, is known (Couzinet et al., 1988; Shoham et al., 1991). Oocytes obtained from in-vitro fertilization (IVF) cycles in hypogonadotrophic women treated with purified or recombinant FSH (recFSH) alone, showed reduced fertilization rates compared with the same patients treated with combined FSH and LH, indicating that the LH depleted environment yields oocytes with a reduced potential for further development (Balasch et al., 1995). Furthermore, there is experimental evidence from studies in sheep which show that inhibited oestradiol biosynthesis during oocyte maturation has a detrimental effect on the developmental competence of oocytes (Moor et al., 1996).

Further evidence for LH requirements during the follicular phase derives from studies in macaque monkeys in which the LH was suppressed using a gonadotrophin-releasing hormone (GnRH) antagonist. They showed normal follicular growth and oocyte yields, and a higher fertilization rate when recFSH was used, compared with a combination of recFSH and recombinant LH (recLH) (Zelinski-Wooten et al., 1995). However, the absence of recLH had a detrimental effect upon cryosurvival, embryo development and implantation after cryopreservation and thawing (Weston et al., 1996). In women undergoing IVF, purified and (more recently) recFSH alone are being used as the stimulants in conjunction with a GnRH analogue (GnRHa). In the majority of such women, there is some endogenous LH activity which might be sufficient for normal follicular growth and oocyte development. However, it has been shown that purified FSH induces lower oestradiol in the circulation than human menopausal gonadotrophins (HMG) (Fleming et al., 1996; Fried et al., 1996), and that approximately one-third of women undergoing IVF demonstrated suppression of LH during the follicular phase (assessed by immunoassay), which was sufficient to reduce the testosterone and oestradiol concentrations found in the follicular fluids (Fleming et al., 1996). The effects of suppression of LH during the follicular phase of the stimulation cycle upon cryosurvival, embryo development and implantation after cryopreservation have not been previously reported in humans.

This study examines the impact of low circulating LH concentrations during the stimulation cycles treated with GnRHa and purified FSH, upon the survival, developmental potential and implantation of cryopreserved embryos. During the examination period cycles were treated with either HMG (containing LH activity) or purified FSH (containing negligible LH) and the circulating LH concentrations were examined in stored plasma samples from the mid-follicular phase of the treatment cycles.

Materials and methods

All transfers of cryopreserved embryos between February 1995 and November 1996 were analysed. They derived from 250 stimulated IVF cycles in 156 women, all of which used the long GnRHa protocol initiated on day 21 of the previous cycle, followed by stimulation using a starting dose equivalent to 225 IU FSH per day.

During the examination period, the laboratory protocol routinely
employed HMG as the follicular stimulant, but restricted availability of this product necessitated increased use of purified FSH.

The main indications for treatment were tubal blockage and unexplained infertility, and the distributions were similar in both treatment groups (HMG group; 68% tubal and 18.5% unexplained; purified FSH group; 61.2% tubal and 26.4% unexplained).

**Embryology, cryopreservation and transfer protocol**

During the stated period, all embryos were cryopreserved at the pronuclear stage of development, and a constant methodology for cryopreservation and thawing (Lassalle et al., 1985) was employed. Embryo transfers were effected ~24 h after thawing, when the embryo development rate was calculated and recorded for each embryo (Cummins et al., 1986). In women (n = 148) with normal menstrual rhythm (87% of cycles), transfers were effected in natural cycles following the LH surge detected by urinary LH dipstick methodology. The first day of LH surge detection was considered to be the day of the LH surge, and thawing was effected 2 days later and transfer the following day. In cases where the menstrual rhythm was disturbed, or problems with LH surge determination existed, the patients were treated with GnRH and exogenous gonadotropins to obtain oestradiol and progesterone profiles as in ovulation induction cycles. In these cases, embryo transfer was effected by reference to administration of the luteinizing HCG.

The number of embryos thawed, the number surviving, the embryo development rates, and the number of embryos transferred were recorded in all cases. The implantation rates of transferred embryos and the pregnancy rates were analysed.

**The embryo development rate**

The number of cells observed and the time in culture were used to calculate an estimate of the growth rate of each embryo and the mean of these was recorded for the embryos transferred in each case.

**Definition of clinical pregnancy and implantation rates**

Clinical pregnancy rate was defined as the number of clinical pregnancies (positive fetal heart at ultrasound examination) diagnosed during the first trimester, expressed as a percentage of the cases in which embryo transfer was effected.

Implantation rate was defined as the number of gestational sacs with fetal heart identified on ultrasound scan, expressed as a percentage of the number of embryos transferred.

**Designation by LH**

Cycles treated with HMG (Pergonal; Serono UK Ltd, Welwyn Garden City, UK) were presumed to have been exposed to LH. Cycles treated with purified FSH (MHP; Metrodin-HP; Serono UK Ltd) were divided according to the mean circulating LH concentration determined on cycle days 6 and 7. The samples were stored from all cycles and were assayed for LH in a single batch. The concentrations of LH were assayed using a specific fluorimunoassay (DELFIAB, Wallac UK, Milton Keynes, UK) with interassay variation of 6.9% and intra-assay variation of 3.9%.

Depending on the stimulant and LH concentrations, the cycles were divided into three groups: group 1, HMG as the stimulant; group 2, purified FSH as the stimulant and where mid-follicular phase LH >1.0 IU/l; and group 3, purified FSH as the stimulant and where mid-follicular phase LH ≤1.0 IU/l.

The threshold concentration of LH (1.0 IU/l) was used because mean follicular phase concentrations below this value had been shown to reduce follicular fluid testosterone and oestradiol biosynthesis in a previous investigation (Fleming et al., 1996), and because the assay remains reliable at this point.

**Statistical analyses**

The results are summarized as totals per group, and also as means and SD per case, which were compared using the Mann–Whitney test. The limited range of values in these analyses did not describe true normal distributions, but transformation was not a viable alternative. Group results were compared using contingency table analyses.

**Results**

During the examination period, 351 consecutive embryo transfers with cryopreserved embryos were effected. They derived from 173 cycles treated with HMG (mean age at time of treatment: 32.5 years) and 77 cycles treated with purified FSH (mean age: 33.1 years). Of the latter patient cycles, 21 showed mean follicular phase LH concentrations ≤1.0 IU/l (i.e. suppressed LH, group 3), with 56 cases in the ‘normal’ LH group (>1.0 IU/l, group 2).

The numbers of embryo transfers, embryos thawed, survived and replaced in each group are shown in Table I. There was no difference in the proportions of embryos surviving the cryopreservation and thawing processes in any of the groups, and no differences in the numbers of embryos transferred per case. These data indicate that there was no impact of the suppressed follicular phase LH concentrations upon apparent embryo viability.

Table II shows that there was no difference in the embryo development rates recorded in the three groups of patients. Similarly, the proportions of embryos replaced, the pregnancy rates and the implantation rates were not influenced by the follicular phase circulating LH activity.

**Discussion**

The role of suppressed follicular phase LH in embryo development following cryopreservation, was examined by detailing the proportion of embryos surviving the thawing process, individual embryo developmental rates, proportions of embryos transferred, and implantation rates in patients treated with HMG and in patients treated with purified FSH who showed either normal or ‘suppressed’ LH concentrations in the follicular phase. The criteria used for examining the effects of low LH during the follicular phase have been shown previously to influence reproductive steroid biosynthesis, and the incidence of suppressed LH (<1.0 IU/l) in this study was similar to that reported previously (Fleming et al., 1996). These detailed examinations of the performance of cryopreserved embryos following stimulated cycles failed to detect any effect of the degree of LH suppression upon embryo developmental performance.

Most of the available evidence shows that FSH alone is sufficient to induce follicular growth. However, the effect of LH deprivation on oocyte development, fertilization and development potential of embryos is only beginning to be explored. The theoretical absence of LH as in hypogonadotropic hypogonadism, or the profound suppression of LH as occurs in some cycles treated with GnRHα, results in reduced oestradiol biosynthesis and secretion (Couzinet et al., 1988; Shoham et al., 1991; Fleming et al., 1996). The limited data
available in humans from examination of oocytes from IVF cycles without LH suggests that oocyte development and fertilization rates are compromised by an absence of LH (Balasch et al., 1995).

The studies of profound LH suppression with a GnRH antagonist in monkeys (Zelinsky-Wooten et al., 1995; Weston et al., 1996) showed a compromised ability of cryopreserved embryos to survive the thawing process and also demonstrated a negative influence upon their developmental potential. This implies that the reduced follicular phase LH concentration was influencing oocyte and embryo ultrastructure, or a sequence of ultrastructural changes, to a degree that rendered them vulnerable to the cryopreservation processes. These data are consistent with the clinical cases of hypogonadotrophic hypogonadism described above. However, this does not appear to be paralleled in clinical practice where LH suppression by GnRHα may be profound enough to influence oestriadiol biosynthesis, but it appears insufficient to influence embryo potential after cryopreservation, using the pronuclear cryo-methodology described here.

It is clear that in most cases, the use of GnRHα in normal women to facilitate control of the stimulation cycle is reasonable, as there is sufficient LH in the circulation to allow follicular development, and relatively normal oocyte and embryo development. Nevertheless, it is possible that the use of this methodology may be detrimental in a small proportion of cases where the LH is suppressed to a degree which influences follicular development. However, it appears that embryo viability after cryopreservation is not affected in most cases.

A parallel prospective study in fresh IVF cycles appeared to confirm that LH suppression influenced oocyte yield, fertilization rates and embryo yield, but had no effect upon the proportion of embryos attaining blastocyst formation (Lloyd et al., 1997). This suggests that once the oocyte is fertilized, the developmental potential is not influenced by the degree of LH suppression during the follicular phase.

**References**


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