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REPRODUCTIVE MEDICINE

Elevated progesterone in GnRH agonist down regulated in vitro fertilisation (IVFICSI) cycles reduces live birth rates but not embryo quality

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Abstract
Objective To assess the impact of pre-hCG elevated progesterone on live birth outcomes during GnRH agonist long down regulated protocol assisted reproduction cycles.
Design Retrospective cohort study.
Setting Single Centre Private IVF Clinic.
Patients A total of 582 consecutive cycles of IVF/ICSI in 2003.
Interventions All patients underwent a long down-regulation protocol, controlled ovarian stimulation and IVF/ICSI. Serum progesterone concentrations were measured just prior to HCG administration. 253 patients were followed to 2009 for outcomes of their frozen embryo cycles.
Main Outcome measure Live birth rate in fresh and frozen cycles.
Results Patients in the upper quartile pre-hCG progesterone concentration (≥5.4 pmol/L) had a higher final estradiol level, more oocytes collected and more usable embryos, when compared to those with lower quartiles. They also had lower live birth rates per cycle started (21.9% vs. 15%, $P < 0.05$). However, live birth rates from frozen embryo cycles were not significantly different between the groups.
Conclusions Pre-hCG progesterone elevation leads to lower live birth rates in stimulated IVF cycles. Live birth rates achieved with frozen embryos in the high progesterone cycles suggest, that pre-hCG progesterone elevation negatively affects endometrial receptivity without adversely affecting embryo quality.

Keywords GnRH agonist · Progesterone · IVF · Frozen embryos · Live birth rates · Endometrial receptivity

Introduction

The effect of premature progesterone elevation on IVF outcomes represents a subject that has attracted much controversy. Furthermore, the underlying pathophysiology has been a subject for debate.

Despite pituitary suppression using gonadotropin-releasing hormone agonists (GnRH-a), reports of elevated serum progesterone levels prior to HCG administration surfaced in the early 1990s [1–3]. This phenomenon has been referred to as 'premature luteinisation'. Several studies have reported a negative effect of progesterone elevation on pregnancy rates [4–8]. On the other hand, some studies have found no such effect [9–12]. It is uncertain whether premature progesterone elevation adversely affects endometrial receptivity or embryo/oocyte quality [13–15].

Early studies suggest dysfunctional folliculogenesis as the cause of premature luteinisation, often in poor responding patients [11, 12]. More recent evidence points to an accumulation of progesterone as a result of multiple normally developing follicles [16].

Some of the findings were presented as an oral presentation at the Fertility Society of Australia Annual Scientific Meeting in Sydney, Australia; and as a poster presentation at the Conjoint Meeting of the American Society for Reproductive Medicine and Canadian Fertility and Andrology Society in Montreal, QC, Canada.

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The purpose of this study is first to evaluate whether high serum progesterone concentrations pre-HCG administration adversely affects live birth rates. Second, by assessing frozen embryo transfers from embryos generated in high progesterone cycles, the study aims to assess the quality of these embryos.

Materials and methods

A retrospective single centre cohort study was conducted, with 582 consecutive IVF or ICSI treatment cycles from January 2003 to December 2003. Frozen embryo transfer cycles occurred between 2003 and 2009. All patients were only included once for the analysis.

Patients underwent a long down-regulation protocol using nafarelin acetate nasal spray. The following groups were excluded: (a) patients undergoing donor oocyte treatment, (b) women diagnosed with hypogonadotrophic hypogonadism, (c) patients using other GnRH analogues or GnRH antagonists, and (d) patients presenting from affiliated clinics for pre-implantation genetic diagnosis.

The study was approved by the Research and Development Committee of IVFAustralia.

The standard treatment protocol was as follows: pituitary down-regulation was achieved with GnRH agonist (Nafarelin 200 μg intranasal twice a day, Synarel, Searle Australia) from the mid-luteal phase in ovulatory patients and after using medroxyprogesterone acetate (Provera, Pharmacia Australia) 10 mg daily for 5–7 days in anovulatory patients. 12 days later, a hormonal assessment was performed. A serum estradiol level <200 pmol/L was accepted as adequate suppression of ovarian activity as per IVFAustralia's protocol. Once this was achieved, ovarian stimulation was initiated with rFSH (Gonal F®; Serono Australia, or Puregon®; Organon, Australia) using an individualized dose (100–450 IU) according to age, and previous response. Ultrasound examinations and serum oestradiol concentrations commencing on day 7 of stimulation were used to monitor the ovarian response, with adjustment to the dose of FSH administered if necessary. When at least two follicles had reached a diameter of >18 mm, 10,000 IU of HCG (Profasi®; Serono Australia; or Pregnyl, Organon Australia) was administered to induce final follicular maturation. Oocyte retrieval was performed 36–38 h later by vaginal ultrasound-guided follicle aspiration. A maximum of two embryos were transferred on day 2, 3 or 5 after retrieval. Luteal phase support was given by daily administration of vaginal progesterone pessaries (200 mg per day, Orion, Australia) or HCG (Pregnyl 1,500 IU in 3 doses at 72-h intervals). IVFAustralia does not have a cancellation policy for cases of progesterone elevation prior to oocytes retrieval.

Frozen embryo transfer cycles were either performed as a natural cycle (timed according to spontaneous ovulation) or as a hormone assisted cycle. For the hormone assisted cycles, Progynova (estradiol valerate, 4 mg/day) was administered from day 4 of the menstrual cycle. An ultrasound scan was performed on day 12 of the cycle to assess endometrial development. An endometrial thickness of ≥7 mm was considered suitable for embryo transfer. If this was not achieved at the first ultrasound, the dose of Progynova was increased and the ultrasound was repeated 3–6 days later. Once endometrial development was adequate, progesterone pessaries (per vagina) were started at a dose of 100 mg twice a day.

The frozen embryos were thawed and in most cases cultured over night. Intact embryos were transferred irrespective of cell division on the day after thawing. Pregnancy tests were performed 16 days after ovulation or progesterone commencement. In hormone-assisted cycles, both progesterone and the Progynova were continued until 12 weeks gestation if pregnant.

Blood samples were collected as part of the routine monitoring on stimulation just prior to HCG administration (late follicular phase). The final progesterone measurement (final P4) was taken within 36 h prior to HCG administration in most cases. Sera were immediately analysed for estradiol, LH and progesterone. The assay used for LH, estradiol and progesterone measurement was a commercially available ACS180 automated chemluminescent immunoassay system (Bayer diagnostics, Australia). The sensitivity values for progesterone, estradiol and LH were 0.35 nmol/L, 36.7 pmol/L, and 0.07 IU/L respectively. The intra and inter assay variations for LH, were <5.6 and <7.2%, for progesterone <10 and <12% and for estradiol, <9.4 and <9.8%, respectively, in the range of concentrations measured in this study.

The primary outcome was live birth rate. A clinical pregnancy was defined as foetal heart motion seen on ultrasound. A miscarriage was defined as a clinical pregnancy loss before 20 weeks gestation. The number of usable embryos was defined as the number of embryos that were suitable for transfer or cryopreservation. Embryos were assessed on the basis of developmental stage and the level of cell fragmentation, for example on day 2 after insemination embryos with 4 cells or more and less than 20% fragmentation were deemed to be suitable for fresh transfer and those with less than 10% suitable for freezing. Obstetric outcomes were confirmed with the patients. The multiple pregnancy rate was calculated as the number of multiple births divided by the total number of births after 20 weeks gestation.

Comparisons of means in normally distributed parameters were made using Student's *t* test. Chi-squared analyses were performed when data were presented in the form of a

percentage or success/failure outcomes. Significance was recorded for tests with $P < 0.05$

Results

A total of 582 patients aged 20–45 years (mean 33.7 years) were included in this study. They underwent consecutive IVF/ICSI cycles from January to December 2003. Every patient was included once only. One patient was lost to follow-up (patient re-located overseas), and was included in all analyses except for the live-birth data. Quartiles were used to differentiate final progesterone concentrations as previous studies have used this criterion [18]. Entry demographics such as age and attempt number were comparable (Table 1).

Patients in the upper quartile of final progesterone concentrations (≥5.4 pmol/L) were found to have significantly higher final estradiol concentrations, more oocytes collected and more embryos suitable for freezing (Table 2).

The average number of embryos transferred was not statistically significantly different. In quartile 4 (Progesterone ≥ 5.4 pmol/L), live birth pregnancy rates per cycle started were significantly reduced (Table 3). Differences in clinical pregnancy rates and miscarriage rates did not reach statistical significance. Table 4 outlines the reasons for cycles that did not result in an embryo transfer.

Significantly more patients in quartile 4 had frozen embryos. Between 2003 and 2008, 253 patients with embryos generated from the above treatment cycles returned for subsequent treatment. 233 patients had frozen embryo transfers. The average number of embryos transferred, clinical and live birth rates did not differ significantly (Table 5).

Discussion

The results of this study suggest that elevated serum progesterone concentration prior to HCG triggering seems to lead to lower live birth rates per cycle started. At the same time, frozen embryos from high progesterone cycles result in similar live birth rates compared to embryos generated from cycles with normal pre-HCG progesterone levels.

Venetis published a meta-analysis published in 2007, which highlighted the controversy surrounding pre-HCG progesterone elevation negatively affecting pregnancy rates [17]. This meta-analysis did not find an association between a pre-HCG increase in Progesterone and decreased clinical pregnancy rates. This meta-analysis has been criticised because of the different P4 cut-offs and the different assays used. Recently, the first prospective randomised controlled trial demonstrated a reduction in pregnancy rates with increased pre-HCG P4 conentrations [16]. Further to this, Bosch has the largest retrospective trial (over 4000 IVF cycles) reporting a negative effect (12% reduction in ongoing pregnancy rate) of raised pre-HCG P4 concentrations on ongoing pregnancy rates [4]. In summary, the recent evidence does suggest that elevated pre-HCG P4 levels reduce clinical pregnancy rates.

The underlying pathophysiology of pre-HCG progesterone elevation is also controversial. The term premature luteinisation has been used for this phenomenon [1–3], which infers a luteinising change in the growing follicles.

Table 1 Patient demographics

	Quartile 1–3 P4 < 5.4 pmol/L (437 patients) ± SEM	Quartile 4 P4 ≥ 5.4 pmol/L (117 patients) ± SEM	*P* value
Average age (years)	33.6 ± 0.2	33.7 ± 0.4	NS
Average IVF attempt number	2.03 ± 0.11	1.92 ± 0.15	NS
Average amount of FSH used (IU)	2054 ± 46	2103 ± 76	NS

Table 2 Results of controlled ovarian stimulation

	Quartile 1–3 P4 < 5.4 pmol/L ± SEM	Quartile 4 P4 ≥ 5.4 pmol/L ± SEM	*P* value
Average final estradiol concentration (pmol/L)	6,551 ± 174	8,294 ± 287	<0.0001
Average final progesterone concentration (pmol/L)	3.41 ± 0.15	6.84 ± 0.11	<0.0001
Average number of oocytes collected	10.0 ± 0.3	12.7 ± 0.6	<0.0001
Average number of embryos transferred	1.43 ± 0.03	1.47 ± 0.05	NS

Table 3 Pregnancy outcomes

	Quartile 1–3 P4 < 5.4 pmol/L	Quartile 4 P4 ≥ 5.4 pmol/L	*P* value	Odds ratio [95% confidence interval]
Live birth rate per transfer	99/360 (27.5%)	22/117 (18.8%)	NS	0.611 [0.364–1.025]
Live birth rate per cycle started	99/435 (21.9%)	22/147 (15.0%)	0.044	0.597 [0.36–0.99]
Clinical pregnancy rate per transfer	131/361 (36.3%)	34/117 (29.1%)	NS	0.719 [0.457–1.131]
Clinical pregnancy rate per cycle started	131/436 (30.0%)	34/147 (23.1%)	NS	0.701 [0.454–1.082]
Miscarriage rate	29/131 (22.3%)	10/34 (29.4%)	NS	
Miscarriage rate per cycle started	29/435 (6.7%)	10/147 (6.8%)	NS	
Proportion of patients with embryos frozen	227/436 (52.1%)	92/147 (62.6%)	0.027	

Table 4 Reason for no transfer

Reason for no transfer	Quartile 1–3	Quartile 4
Failed stimulation (poor response)	19 (25%)	12 (40%)
Failed oocyte retrieval	3 (4%)	0
Failed fertilisation	25 (33%)	2 (7%)
Failed embryo development	5 (7%)	2 (7%)
Freeze all (risk of OHSS)	23 (30%)	14 (46%)

This may occur with or without a rise in LH. Venetis et al. [17] propose a different aetiology. In their meta-analysis, raised pre-HCG progesterone was associated with an increased number of ovarian follicles and higher E2 levels, findings that are in keeping with the current study. They suggest that the elevated progesterone may be a result of an excess number of follicles, each one producing a normal amount of progesterone, all adding together to produce the elevation in progesterone levels [17].

This study is unique because it evaluates the outcome of frozen embryo transfer cycles, as a measure of embryo quality in a different implantation setting. The study shows similar live-birth rates in patients with frozen embryos from raised pre-HCG progesterone cycles when compared to those embryos from normal cycles. This would support the theory that premature progesterone exposure does not affect the quality of the embryos, and more likely decreases endometrial receptivity.

Melo et al. [19] published a study looking at progesterone elevation in donor oocyte cycles and the subsequent implantation outcomes for the recipient cycles. This study retrospectively looked at 120 women who donated twice, with premature luteinization (serum LH ≥ 10 IU/L) in the first donation cycle and no premature luteinization in the following one, acting as its own control. There was no difference in pregnancy rates between the groups (55.7 vs. 54.4%, respectively). The authors concluded that premature luteinization does not appear to have a negative impact on ongoing pregnancy rates in the oocyte-donation programme. This would suggest that oocyte quality is not affected [19].

Furthermore, Fleming and Jenkins [20] suggest that the raised progesterone levels may lead to asynchrony between endometrial and embryo development, thus affecting pregnancy rates.

The limitations of the current study lie in the retrospective nature of the trial. Potential sources of bias include heterogeneity of stimulation protocols and cancellation criteria. This study was also unable to evaluate an association between the duration of controlled ovarian stimulation or the duration of progesterone elevation, and

Table 5 Results of frozen embryo transfer cycles

	Quartile 1–3 P4 < 5.4 pmol/L	Quartile 4 P4 ≥ 5.4 pmol/L	*P* value
Number of live birth pregnancies (live birth rate/transfer %)	51 (30.7%)	20 (29.9%)	NS
Number of patients with embryos frozen	227	92	
Number of patients returning for FET	179 (78.9%)	74 (80.4%)	NS
Number of patients that had frozen embryos transferred	166	67	
Number of frozen embryos transferred	222	86	
Mean number of embryos transferred	1.34	1.28	NS
Number of clinical pregnancies (clinical pregnancy rate/transfer %)	61 (36.7%)	26 (38.8%)	NS
Number of miscarriages (miscarriage rate %)	10 (16.4%)	6 (23.1%)	NS
Number of multiple births (multiple birth/birth %)	5 (9.8%)	0 (0.0%)	NS

pregnancy outcomes. Potential confounders such as female age and cycle number and dose of FSH were comparable between treatment groups. The number of oocytes retrieved was significantly increased in quartile 4, which may well be a confounding factor. On the other hand, it supports the physiological hypothesis, that it is the sum total of the increased number of follicles produced that causes progesterone to rise.

The lack of statistical significance in the live birth rate per transfer raises some questions. One reason may be that the sample size is not large enough, when all the cycles that did not reach transfer, were excluded. Certainly, the proportion of pregnant to non-pregnant patients (odds ratios) was similar when comparing live birth rates per transfer and per cycle started, supporting this argument. Another explanation could be the higher rate of freeze-all cycles due to the risk of OHSS.

Assuming that pre-HCG progesterone elevation has a negative effect on live-birth rates, then this in itself may represent a cause of implantation failure. Current recommendations for preventing this phenomenon include preventing an LH surge and using less FSH to recruit fewer follicles. Administering HCG earlier in the cycle may also prevent the progesterone rise. The current study shows that freezing of embryos may be an alternative method to achieve successful pregnancies.

Recently published data from Shapiro et al. [21] concluded that in cycles with elevated preovulatory progesterone, the probabilities of implantation and ongoing pregnancy are increased if all 2PN oocytes are cryopreserved and subsequently thawed and cultured to the blastocyst stage before transfer.

Another study has suggested that a blastocyst transfer (Day 5) may not be associated with decreased pregnancy rates in the presence of premature progesterone elevation [22]. The study presented here mainly consisted of cleavage stage embryo transfers. Whether a Day 5 embryo transfer can overcome the effects of premature luteinisation needs further evaluation.

Conclusion

In conclusion, this study supports the hypothesis that pre-HCG progesterone elevation negatively affects live-birth rates, however, this is not due to reduction of embryo quality, as frozen embryos from high pre-HCG progesterone cycles have good potential. A larger prospective trial may be of value in further delineating how best to treat the clinical problem of pre-HCG progesterone elevation.

Conflict of interest The authors declare there is no conflict of interest.

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