Letters to the Editor

Preovulatory rise in progesterone

Dear Sir,

We read with great interest the article by Eldar-Geva et al. (1998). In the late follicular phase of gonadotrophin-releasing hormone (GnRH) agonist/human menopausal gonadotrophin (HMG)-stimulated cycles they showed a concomitant increase of serum progesterone and luteinizing hormone (LH), both of which were reversible by the administration of dexamethasone. Based on these findings, they concluded that part of the follicular phase progesterone is of adrenal origin. Their results seem to support the notion that high oestrogen concentrations may cause discrete changes in the hypothalamic–pituitary adrenal axis and in adrenal enzymatic activity as part of a complex ‘cross-talk’ between the hypothalamic–pituitary–ovarian and the hypothalamic–pituitary–adrenal axes. This proposal is feasible, although there are still some other aspects which need to be given further consideration.

Bognar et al. (1991) measured follicular fluid oestradiol, progesterone and testosterone concentrations in patients involved in an in-vitro fertilization (IVF) and embryo transfer programme. Of the three steroids, progesterone showed the best correlation with follicular maturation. A significant difference was established in progesterone content of first versus second class, as well as of first versus third maturity class follicles. The progesterone content of the mature follicles significantly exceeded that of the immature ones. Similarly, a significant difference was found in the progesterone/oestradiol ratios of the immature and mature follicles. The follicular progesterone probably originates from granulosa cells of growing follicles and can be regarded as the most likely source of the rise in serum progesterone concentration.

In our earlier study (Bódis et al., 1993), we measured the progesterone, oestradiol, serotonin, noradrenaline and dopamine contents of follicular fluid samples obtained from 35 patients undergoing IVF and embryo transfer. Ovarian stimulation was performed using combined suppression–stimulation therapy. The GnRH agonist triptorelin (Decapeptyl; Ferring, Kiel, Germany) was used in a long protocol. Stimulation was carried out with individual dosages of HMG (Humegon; Organon, Oberschleisheim, Germany), varying from two to six 1 ml ampoules daily depending on the follicular maturation. Ovulation was induced by injection of 10 000 IU human choricron gonadotrophin (HCG, Predalon; Organon), and aspiration of follicular fluid was performed 36 h later by ultrasound-guided vaginal puncture. Significantly higher progesterone and noradrenaline concentrations have been found in human follicular fluid from cycles in which the oocyte cleaved and resulted in pregnancy, compared with follicular fluid containing uncleaved oocytes (Bódis et al., 1993). These results suggest that the higher concentration of progesterone and noradrenaline within follicular fluid may be important for oocyte maturation and its ability to become fertilized, cleave and implant. This progesterone is clearly of granulosa cell origin.

In other experiments, we studied the progesterone secretory characteristics of human granulosa cells using an in-vitro cell superfusion model (Török et al., 1998). It was found that the basal progesterone secretion is pulsatile, even with no stimulation. After stimulation with LH there is a rapid, but slight increase in the steroid values, followed by a delayed and also pulsatile definitive increase of progesterone concentrations ~30 min later. Hence, it seems likely that human granulosa cells store a small quantity of progesterone which can be released by LH. The delayed progesterone increase is due to the de-novo synthesis of the hormones after gonadotrophin stimulation, which takes ~60 min.

Existence of ‘cross-talk’ between the adrenal and ovarian axes might be relevant to reproductive events. Nevertheless, a common feature of adrenal and ovarian steroid synthesis is the use of common precursors and the two glands also share some of their products; one of these is progesterone. Dexamethasone could have direct or indirect effects on both loci. Further delineation of the source of the late follicular phase progesterone rise in the serum seems necessary. Whether it is of primarily ovarian and secondarily adrenal origin remains to be established.

References


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Letters to the Editor

Dear Sir,

We thank Büdis et al. for their interest in our article on the origin of serum progesterone during the follicular phase of menotrophin-stimulated cycles (Eldar-Geva et al., 1998). Indeed, none of the papers cited by them in their letter seems to contradict our conclusion that at least some of the serum follicular phase progesterone appears to be of adrenal origin.

Obviously, we agree that at the time of egg retrieval for in-vitro fertilization (IVF), ~36 h after administration of human chorionic gonadotrophin (HCG), follicular fluid progesterone originates from the granulosa cells of ovulating follicles. HCG directly induces luteinization in the granulosa cells, namely progesterone secretion. Serum progesterone at this time is usually at least four times higher than the concentration before HCG administration (Hsiung et al., 1990). In spontaneous cycles, serum progesterone 36 h after the onset of the luteinizing hormone (LH) surge is more than twice as high as the concentration reached at the time of the initiation of the surge (Hoff et al., 1983) We completely agree with the notion that the most of the serum progesterone at this stage is of ovarian origin.

Our study targeted the events before HCG administration. We did not cite in our paper the results of many studies measuring progesterone and oestradiol concentrations in follicular fluid obtained during egg retrieval. These studies are not relevant to the aim of our study, since they deal with events occurring after the administration of HCG.

Two more papers could be added to support our hypothesis that oestradiol stimulates the hypothalamic–pituitary–adrenal axis activity in humans. Using short-term oestradiol stimulation in young healthy men, Kirschbaum et al. (1996) also reported that oestrogen enhanced human hypothalamic–pituitary–adrenal axis activity. At least two molecular mechanisms could be involved in oestrogen up-regulation of the hypothalamic–pituitary–adrenal axis activity. The human corticotrophin-releasing hormone (CRH) gene contains five oestrogen-responsive elements (EREs) within its 5′ flanking region. Specific oestrogen receptor- and ERE-mediated enhancement of the human CRH gene promoter was observed using reporter gene constructs (Vamvakopoulos and Chrousos, 1993). Thus, oestradiol may directly stimulate CRH synthesis and its subsequent secretion. Another possible mechanism suggested in our paper is oestrogen-induced decrease in the inhibitory effect of glucocorticoid feedback on CRH and adrenocorticotrophic hormone (ACTH) secretion, as previously shown in rats (Pfeiffer et al., 1991; Burgess and Handa, 1992).

Nevertheless, as we wrote in our discussion, other mechanisms, such as the direct effects of dexamethasone, ACTH or CRH on granulosa cells could exist. Obviously, we agree that the exact mechanism responsible for the increase in late follicular phase serum progesterone remains to be established.

References


Talia Eldar-Geva

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Sperm separation for sex selection

Dear Sir,

In a recent paper Rose and Wong (1998) present evidence that the use of semen which had been processed by the human serum albumin (HSA) method (Ericsson, 1994) of separating X-and Y-chromosome bearing spermatozoa in artificial insemination lead to a raised birth sex ratio. Semen samples from seven healthy donors also underwent the HSA semen separation method. However, the percentage of Y-chromosome bearing spermatozoa in these samples was found not to have been increased. This cannot be due to inactivation of X-bearing spermatozoa, as suggested by Rose and Wang, because Ericsson (1994) reported that using a larger proportion of processed semen, together with ovulation induced by clomiphene citrate, resulted in an increase in female births.

I recently published (Bernstein, 1996) a short notice recounting experimentation at a German agricultural station ‘Mariensee’ which duplicated the results of the Ericsson method of Y spermatozoa enrichment. Actually, Thornmählen (1973) separated spermatozoa according to their specific gravity; insemination with light semen raised the birth sex ratio significantly for his rabbits, whereas insemination with heavy spermatozoa lowered the birth sex ratio. However, the semen treatment was only successful if the males had been used only once in 2 weeks; no separation was possible with semen obtained every 2 days.

If the husbands whose semen was used for the artificial insemination of the patients of Rose and Wong were asked to not to have an ejaculation for about a week whereas the seven, probably younger, sperm donors may well have had an ejaculation every other day, then the findings by Thornmählen explains why the donor semen showed no enrichment of Y-chromosome bearing spermatozoa after undergoing the Ericsson semen enrichment procedure.
Dear Sir,

Thank you for the opportunity to reply to Dr Bernstein’s letter. As stated in our paper, the donor husbands were asked to abstain from all sexual activity for 4–5 days prior to treatment. The male control volunteers were given exactly the same advice. Therefore, there is no reason to believe that there was any systematic difference between the periods of abstinence of the two groups.

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A. Wong
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Dear Sir,

The study by Rose and Wong (1998) confirms our work (Wang et al., 1994) and that of others (Moore and Gledhill, 1988; Vidal et al., 1993) using fluorescent in-situ hybridization (FISH) technology, which demonstrated that simple sperm selection techniques (albumin columns) do not significantly alter the X to Y sperm ratio. Indeed, the notion that sex selection using albumin gradients is due to enrichment of Y spermatozoa should finally be put to rest, as there is no evidence to support the contention.

However, their most important conclusion is that the clinical programme utilizing this sperm preparation procedure did result in a biased ratio of offspring in favour of more males. Irrespective of the recognized weaknesses of the study (miscarriages, ectopic pregnancies and lost patients), the claim stretches credibility and includes a sex-discrepant twin pregnancy, which is counted as a male, presumably on the basis that the couple in question got the boy they desired (as well as a girl!). There were 16 male babies born and four female babies. Based on these figures, and utilizing the statistical method employed in their paper (Moore and Gledhill, 1988), the result is not significant (P = 0.05) and the conclusion remains unproven. Until the results of properly controlled trials with considerably more babies born, are available, such a sex selection programme should be considered as experimental, with a 50/50 chance of correct prediction.

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Sex ratios following the use of the Ericsson method of sex selection, and following ICSI

Dear Sir,

Rose and Wong (1998), like other users of the Ericsson system of sex preselection (Beernink et al., 1993; Stephens, 1997), claim substantial success in spite of the reported failure of the system to alter the proportions of X- and Y-bearing spermatozoa (Flaherty et al., 1997; Richards et al., 1997). Indeed, Ericsson (1994) acknowledges this puzzling point. In commenting on it, Richards et al. (1997) write of the possibility that sperm samples are altered during the treatment in some way to allow the female reproductive tract to select for specific sperm types at rates unrelated to the ratio in the inseminated sample. The presence of this latter mechanism has not been documented. In a similar vein, Rose and Wong (1998) wrote of the possible sex-selective deactivation of spermatozoa: ‘Such deactivation is not due to a decrease in sperm motility, but would have to

References


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Dear Sir,

In their letter, Wang et al. write that they wish to use Table II to analyse our results. We reported that there were 15 male births and four female births. According to Table II of the Moore and Gledhill paper, this means that P = 0.004–0.021. We believe that this does just indicate statistical significance. To strengthen the case, more births would be useful, and this is in progress.

It then becomes quite unscientific to complain that the claim stretches credibility, especially as we are only confirming previous results in the matter of obtaining the desired sex. What we all have to do, is first establish the facts and then try to explain them as best we can.

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References


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occur in some other way'. The unexplained success of the Ericsson method is reminiscent of the reported unsought low offspring sex ratio (proportion male) following intracytoplasmic sperm injection (ICSI) (Sarraf et al., 1997) and I suggest that similar explanations may be available for both.

There is very substantial evidence that in mammals (including man), hormone concentrations of both parents around the time of conception somehow affect the sexes of the resulting offspring (James, 1996). On the basis of this hypothesis, I predicted that men exposed to dioxin would sire excess daughters (James, 1996) and that rodents gestated between two females would have a different offspring sex ratio from rodents gestated between two males (James, 1989). Both predictions have since been confirmed (Clark et al., 1993; Mocarelli et al., 1996). It seems reasonable, therefore, to suppose that hormonal manipulation of the sperm samples, rather than enrichment of X- or Y-bearing spermatozoa, is responsible for the Ericsson technique’s success. Similarly one might wonder whether the hormonal manipulation (either of the spermatozoa or of the mother) involved in ICSI (as practised by Sarraf et al., 1997) was responsible for the significant excess of daughters.

I have offered a hypothesis on how hormones have this effect (James, 1997), but regardless of the truth of this hypothesis, it seems incontestable that hormones do have such an effect.

References

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Role of regulatory authorities and safety of the pill
Dear Sir,
Thank you for the opportunity of commenting on G. Benagiano’s editorial which discusses previous publications (Rosenberg et al., 1996; Mills, 1997). It is my impression that Benagiano and Mills are not fully informed about the extent and depth of the scientific debate on the issue, i.e. on the evidence for an increased risk for venous thromboembolism (VTE) and third generation oral contraceptive use. This may apply, too, for the assessment procedure itself. Therefore, I would like to make the following remarks.

Publication of data in a peer-reviewed journal cannot be a precondition for decision-making by the competent authorities. This may lead to an unacceptable delay in actions needed to protect patients’ health. We are aware of a lot of relevant studies on other safety problems, which have never been published and which in the past have been the basis for regulatory actions.

The same is true for the demand that a consensus must be reached first before taking actions. We are aware of some important safety problems, in which the scientific community to my impression, will never reach a consensus.

Evaluation and assessment by impartial scientists has been carried out. There is good reason to assume that the evaluators and assessors working in national authorities are impartial, objective and responsible in every respect. I think this is also true for members of the Committee for Proprietary Medicinal Products (CPMP) which discussed the matter repeatedly, installed an expert group with epidemiologists and pharmacovigilance experts from EU member states and issued three position statements. The last one (dated January 22, 1997) stated that the increase in the observed risk was about two-fold and was statistically significant in three case-control studies (Jick et al., 1995; WHO, 1995; Spitzer et al., 1996). The CPMP and its working parties have their legal basis in European regulations; it is the scientific advisory group working for the European Agency for the Evaluation of Medicinal Products (EMEA), and preparing decisions for adoption by the European Commission. All parties in the oral contraceptive debate, including the companies, have been involved into the discussion since the beginning.

One should be reminded that, in the meantime, a series of various studies on this special matter has been published, three (epidemiological) studies of them close to the date of decision (November, 1995). I agree that data from one single study would not usually be a sufficient basis for decision making.

The Food and Drug Administration (FDA) was not especially cautious in assessing the published data on an increased risk of VTE and third generation oral contraceptives containing gestoden: the FDA has never authorized such oral contraceptives for the US market, and consequently there was no need to re-evaluate the new data and for actions to be taken because of tolerability concerns.

There is no doubt the need for further clarification of so far unexplained details in the risk–benefit assessment of third generation oral contraceptives. In fact, I am not sure whether we will reach a consensus, according to the word’s original
meaning, very soon. In the meantime, may users be on a probably higher risk for a potentially serious adverse drug reaction?

References

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