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Post-ovulatory ageing of the human oocyte and embryo failure

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We carried out a prospective study of 221 healthy women who were attempting pregnancy. During the study, women collected daily urine samples and kept daily records of intercourse. Ovulation and early pregnancy losses were later identified by immunoassays of urinary human chorionic gonadotrophin and steroid metabolites. We have used these data to examine whether the risk of early pregnancy loss was higher with post-ovulatory ageing of the oocyte. 192 pregnancies were ranked by the probability that the oocyte might have aged before fertilization. There was a statistically significant increase in the risk of early loss as the likelihood of oocyte ageing increased ($P < 0.05$). No similar risk was observed for clinical miscarriages. Post-ovulatory ageing of the oocyte prior to fertilization may cause early pregnancy failure in humans as it does in several other mammalian species.

Key words: embryonic death/human/oocyte/pregnancy

Introduction

The oocytes of hamsters, rats, mice, guinea pigs, rabbits and swine undergo changes within 6–18 h after ovulation that make them less likely to be fertilized and, if fertilized, more likely to produce abnormal blastocysts (Lanman, 1968; Butcher, 1976; Longo and So, 1982; Juetten and Bavister, 1983; Perreault, 1992). Among blastocysts that survive, there are apparently no adverse effects.

Similar data in humans are not available. A few studies have suggested that fertilization of old eggs may increase the risk of spontaneous abortion (Guerrero and Lanctot, 1970; Guerrero and Rojas, 1975; Gray *et al.*, 1985, 1995), but these are far from conclusive. We carried out a prospective study of pregnancy among healthy volunteers in which ovulation and early pregnancy loss were defined biochemically (Wilcox *et al.*, 1988). In previous analyses of these data, few factors were found to be predictive of early pregnancy loss (Wilcox *et al.*, 1990; Baird *et al.*, 1991a,b), although there did appear to be a seasonal pattern to loss (Weinberg *et al.*, 1994a). Given the link between aged oocytes and early pregnancy loss in laboratory animals, we sought to explore this question in our data.

Materials and methods

Our study of early pregnancy was conducted among 221 healthy women (ages 21–42 years, mean 30) who were planning to conceive and who had no history of fertility problems. These women were enrolled at the time they stopped using any form of birth control. Women participated for up to 6 months if they did not become pregnant, and for 8 weeks beyond their last menstrual period (LMP) if they became clinically pregnant. During this time, women collected daily first-morning urine specimens, which were frozen and stored for later analysis. Urine collection was complete for 98% of women-days in the study. Women were also asked to record each morning whether or not they had had intercourse in the previous 24 h (without exact time). For analysis, we assumed each intercourse had occurred on the preceding day.

The laboratory, field and statistical methods have been previously described (Wilcox *et al.*, 1985; Baird *et al.*, 1991a,b; Weinberg *et al.*, 1994b; Weinberg and Wilcox, 1995; Baird *et al.*, 1995). The day of ovulation was defined by measures of oestrogen and progesterone metabolites in urine, the ratio of which decreases abruptly with luteinization of the ovarian follicle. This measure of ovulation corresponds approximately to the urinary luteinizing hormone (LH) peak (Baird *et al.*, 1991) and to rupture of the ovarian follicle (Collins, 1983). For detection of pregnancy, we relied on a highly sensitive and specific polyclonal immunoassay (Armstrong *et al.*, 1984) to define a sustained rise of intact urinary human chorionic gonadotrophin (HCG) (Wilcox *et al.*, 1988).

Our biochemical marker of pregnancy identified 199 conceptions. Forty-eight (24%) ended in early loss (i.e. within 6 weeks of LMP), and the remainder survived to become clinically apparent. Of the 151 surviving pregnancies, 15 (10%) failed between the 6th and 24th weeks, and the rest produced live births. Among the 199 conception cycles, 192 (96%) had a measurable day of ovulation and complete intercourse data around the time of ovulation. These 192 pregnancies (which include all 48 early losses and all 15 later losses) are the basis for the present analysis.

According to our estimates of day-specific conception rates, all conceptions could be attributed to intercourse occurring within a 6 day window that ended on the day of ovulation (Wilcox *et al.*, 1995). Effects of delayed fertilization of oocytes would be most obvious among any pregnancies conceived by intercourse after the day of ovulation; however, in our data, no pregnancies could be attributed to intercourse after the day of ovulation. This may reflect a short life of the oocyte (Weinberg and Wilcox, 1995). Therefore, any effects of post-ovulatory ageing in these data would be limited to conceptions resulting from intercourse on the day of ovulation itself. When intercourse and ovulation occur within the same 24 h period, it is possible that the oocyte could age (if only for a matter of hours) if ovulation occurs *before* capacitated spermatozoa are present. Accordingly, our analysis focuses on those conceptions that could have been produced by intercourse on the day of ovulation.

There were 87 conceptions with intercourse on the day of ovulation. For five of these, intercourse had occurred only on the day of ovulation, and not on any other day in the 6 day fertile window. For

Table I. Early pregnancy loss among 192 pregnancies, ranked from lowest to highest probability that conception could have resulted from fertilization of an aged oocyte

Patterns of intercourse during the fertile days of the menstrual cycle								
−5	−4	−3	−2	−1	Day of ovulation	Conceptions	Early losses	% early loss[a]
−	−	−	−	−	O	105	25	24
−	−	−	−	X	X	27	8	30
−	−	−	X	O	X	37	3	8
−	−	X	O	O	X	9	4	44
−	X	O	O	O	X	8	4	50
X	O	O	O	O	X	1	0	67
O	O	O	O	O	X	5	4	
Total						192	48	25

'X' designates intercourse, 'O' no intercourse, and '−' either intercourse or not.
[a]Trend test (two-tailed) for risk of early loss: $P = 0.049$.

these five, we assume the intercourse on day of ovulation was unequivocally the intercourse that produced the pregnancy. For the remaining 82 conceptions, there was at least one act of intercourse earlier in the fertile window (in addition to that on day of ovulation). These earlier days of intercourse make the timing of the ensuing conceptions more ambiguous, by increasing the chance that the pregnancy might have been produced by spermatozoa already present at the time of ovulation.

Even so, these pregnancies are not completely uninformative. We know from previous analysis that the earlier the intercourse occurs during the 6 day fertile window, the less likely it is to produce conception (Wilcox *et al.*, 1995). This gradient of fertility presumably reflects such factors as deterioration of viable spermatozoa in the female reproductive tract over time. Thus, among pregnancies with intercourse both before and on the day of ovulation, the contribution of intercourse before ovulation will be the least when it has occurred earliest in the fertile window.

We ranked pregnancies according to the probability that pregnancy resulted from intercourse on the day of ovulation using information on previous intercourse. This in turn ranked pregnancies by the probability that the oocyte could have deteriorated by the time of fertilization. Thus, conceptions with intercourse exclusively on the day of ovulation had the highest chance of the oocyte ageing before fertilization. Conceptions with intercourse on the day of ovulation and then 5 days before ovulation had the next-highest chance of oocyte ageing before fertilization. The lowest probability of oocyte ageing was among the 105 pregnancies lacking any intercourse on day of ovulation, and therefore for which the fertilizing spermatozoa were already present at ovulation. We assessed the evidence statistically by carrying out a trend test for these ranks, using logistic regression to model the risk of early loss.

Results

Table I shows all pregnancies ranked by the probability that the oocyte could have aged before fertilization. The proportions of early loss range from 24% in the group with lowest likelihood of aged oocytes, to 67% in the group with highest likelihood. A test of trend (two-tailed) was statistically significant ($P = 0.049$). We repeated this analysis for the clinical pregnancy losses occurring after 6 weeks (data not shown). There was no evidence of an association between the likelihood of aged oocytes and the risk of clinical loss, although the small number of clinical pregnancy losses limits this finding.

Table II. Early pregnancy loss among 105 pregnancies with no intercourse on day of ovulation, ranked according to the age of youngest cohort of spermatozoa at conception

Pattern of intercourse during the fertile days of the menstrual cycle								
−5	−4	−3	−2	−1	Day of ovulation	Conceptions	Early losses	% early loss[a]
−	−	−	−	X	O	68	17	25
−	−	−	X	O	O	25	4	16
−	−	X	O	O	O	5	2	40
−	X	O	O	O	O	6	1	29
X	O	O	O	O	O	1	1	
Total						105	25	24

'X' designates intercourse, 'O' no intercourse, and '–' either intercourse or not.
[a]Trend test (two-tailed) for risk of early loss: $P = 0.72$.

The data in Table I raise the question of whether ageing spermatozoa might also have contributed to the observed association. In previous analyses, we had found no association between old spermatozoa and early loss (Weinberg and Wilcox, 1995; Wilcox *et al.*, 1995). We pursued the question further by selecting the 105 pregnancies for which there was no intercourse on day of ovulation, and ranking these pregnancies according to most recent intercourse. This in effect ranks pregnancies by age of the youngest cohort of spermatozoa at the time of fertilization (Table II). With this ranking, there was no evidence of an association between age of spermatozoa and early pregnancy loss ($P = 0.72$).

Discussion

Harmful effects on the conceptus from post-ovulatory ageing of the oocyte are well described in laboratory rodents, but similar effects are less certain in humans. Earlier studies of naturally-conceived pregnancies have been limited by imprecise estimates of ovulation (e.g. basal body temperature) and insensitive measures of pregnancy loss (usually clinical signs and symptoms) (Guerrero and Lanctot, 1970; Guerrero and Rojas, 1975; Gray *et al.*, 1985, 1995). In a review of data from artificially assisted conceptions, Tarin (1966) cited mixed evidence for effects of post-retrieval ageing of human oocytes on embryo development. Although most studies had found reduced capacity for implantation and development of aged oocytes, a few found high pregnancy rates. Tarin (1996) speculated that these discrepancies may be due to protocol differences in laboratory techniques, suggesting some difficulty in extrapolating from in-vitro to in-vivo effects of oocyte ageing.

We used data from our epidemiological study of early pregnancy to explore possible effects of oocyte ageing on embryo viability. This study has the advantage of hormonally defined days of ovulation (using urinary steroid metabolites), explicit days of intercourse (as recorded in daily diaries), and the very early detection of pregnancy by a highly sensitive and specific assay for urinary HCG, all within a context of naturally occurring conceptions among healthy women.

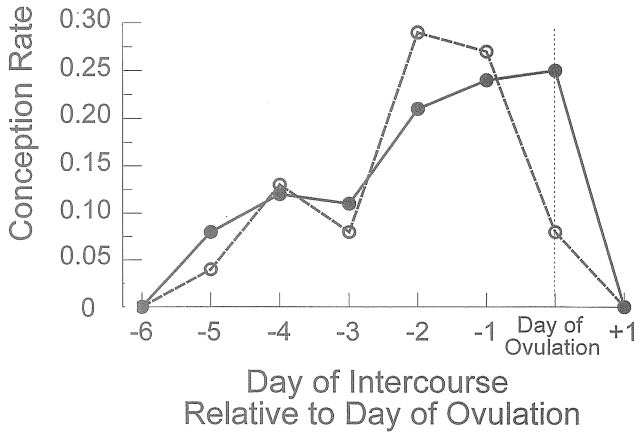

Figure 1. The probability of clinical pregnancy following intercourse on a given day relative to ovulation, shown as 0.75 times the estimate for all conceptions (solid line) and as estimated from clinical pregnancies alone (dashed line). (North Carolina Early Pregnancy Study.)

We defined groups of pregnancies according to the probability that the oocyte might have aged a few hours before fertilization. In these groups, we found evidence that the risk of early pregnancy loss increased with the probability that the oocyte had aged before being fertilized.

Nevertheless, our data have some limitations. They are observational and their analysis necessarily relies on probabilities: we cannot specify which conceptions were produced by old eggs, or which acts of intercourse led to specific conceptions. Furthermore, the numbers are small, and the timing measures are imprecise. The data were collected once a day, which permits only crude assessment of the timing of events for which a matter of hours may be crucial. This lack of precision in the timing of both ovulation and intercourse would be expected to blur any underlying pattern that was present.

Acknowledging these limitations, the data support the hypothesis that post-ovulatory ageing of the oocyte is harmful to embryo survival. This is also consistent with our earlier finding that no pregnancies could be attributed to intercourse after the day of ovulation, when oocytes would be, on average, older than 24 h. While we found no evidence that ageing of spermatozoa was also damaging, there were few pregnancies attributable to aged spermatozoa and so such an effect cannot be ruled out. Also, there could be interactive effects of old spermatozoa and old oocytes, as has been reported in rodents (Smith and Lodge, 1987).

Other factors, such as woman's age, alcohol consumption or smoking habits, might plausibly have an effect on embryo survival. Most women in our study were between the ages of 25 and 35, few were drinkers or smokers, and none of these factors was found to be associated with early pregnancy loss in our study (Wilcox *et al.*, 1990). For example, mothers who conceived a viable pregnancy had a mean age of 29.6 years, while those with an early loss had mean age of 29.7 years. Thus, there was no basis in the present analysis to adjust for maternal age or other factors.

The pattern evident in Table I raises the possibility of an alternative interpretation, namely that fertilization by fresh spermatozoa increases the risk of a defective conceptus. One could speculate that the selective loss of defective sperm in the first day or two after being deposited in the reproductive tract might decrease the chance of defective concepti and hence early losses. This hypothesis may deserve closer scrutiny in the laboratory setting. For the meantime, post-ovulatory ageing of the oocyte seems the more biologically plausible explanation for our results, given evidence from laboratory species that ageing of the oocyte impairs development of the blastocyst.

We previously reported that the probability of intercourse producing conception increases during the 6 days ending on the day of ovulation (Wilcox *et al.*, 1995). If the risk of early pregnancy loss is in fact greater with intercourse on the day of ovulation, then our previous result may not fully describe the fertility pattern for clinically recognized pregnancies. Figure 1 shows day-specific probabilities of conception with intercourse during the 6 day window, estimated in two ways. The solid line is based on probabilities previously estimated for all conceptions (Wilcox *et al.*, 1995), here multiplied by 0.75 to reflect the proportion of conceptions surviving to clinical detection. The dashed line provides recalculated probabilities based on clinical pregnancies alone. While the confidence intervals around all these estimates are very wide, the second set of estimates (shown by the dashed line) suggest that the most likely days for intercourse to produce a clinical pregnancy may be the 2 days before ovulation rather than the day of ovulation itself. Further clarification of these patterns will require larger studies with accurate data on intercourse and ovulation.

In summary, our data suggest that delayed fertilization of the oocyte may increase the risk of very early pregnancy loss. There was no evidence for an increased risk of clinical loss among pregnancies that survived 6 weeks or more.

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References

Armstrong, E.G., Ehrlich, P.H., Birken, S. *et al.* (1984) Use of a highly sensitive and specific immunoradiometric assay for detection of human chorionic gonadotropin in urine of normal, nonpregnant, and pregnant individuals. *J. Clin. Endocrinol. Metab.*, **59**, 867–874.

Baird, D.D., Weinberg, C.R., Wilcox, A.J. *et al.* (1991a) Hormonal profiles of natural conception cycles ending in early, unrecognized pregnancy loss. *J. Clin. Endocrinol. Metab.*, **72**, 793–800.

Baird, D.D., Weinberg, C.R., Wilcox, A.J. *et al.* (1991b) Using the ratio of urinary estrogen and progesterone metabolites to estimate day of ovulation. *Statist. Med.*, **10**, 255–266.

Baird, D.D., McConnaughey, D.R., Weinberg, C.R. *et al.* (1995) Application of a method for estimating day of ovulation using urinary estrogen and progesterone metabolites. *Epidemiology*, **6**, 547–550.

Butcher, R.L. (1976) Pre-ovulatory and post-ovulatory overripeness. *Int. J. Gynaecol. Obstet.*, **14**, 105–110.

Collins, W.P. (1983) Biochemical approaches to ovulation prediction and

detection and the location of the fertile period in women. In Jeffcoate, S.L. (ed.), *Ovulation: Methods for its Prediction and Detection*. Wiley, pp. 49–66.

Gray, R., Kambic, R., Bicego, G. *et al.* (1985) Outcome of pregnancy [letter]. *Fertil. Steril.*, **44**, 554–555.

Gray, R.H., Simpson, J.L., Kambic, R.T. *et al.* (1995) Timing of conception and the risk of spontaneous abortion among pregnancies occurring during the use of natural family planning. *Am. J. Obstet. Gynecol.*, **172**, 1567–1572.

Guerrero, R. and Lanctot, C.A. (1970) Aging of fertilizing gametes and spontaneous abortion. *Am. J. Obstet. Gynecol.*, **107**, 263–267.

Guerrero, R. and Rojas, O.I. (1975) Spontaneous abortion and aging of human ova and spermatozoa. *New Engl. J. Med.*, **293**, 573–575.

Juetten, J. and Bavister, B.D. (1983) Effects of egg aging on *in vitro* fertilization and first cleavage division in the hamster. *Gamete Res.*, **8**, 219–230.

Lanman, J.T. (1968) Delays during reproduction and their effects on the embryo and fetus: 2. Aging of eggs. *New Engl. J. Med.*, **278**, 1047–1054.

Longo, F.J. and So, F. (1982) Transformations of sperm nuclei incorporated into aged and unaged hamster eggs. *J. Androl.*, **3**, 420–428.

Perreault, S.D. (1992) Chromatin remodeling in mammalian zygotes. *Mutat. Res.*, **296**, 43–55.

Smith, A.L. and Lodge, J.R. (1987) Interactions of aged gametes: In vitro fertilization using *in vitro*-aged sperm and *in vivo*-aged ova in the mouse. *Gamete Res.*, **16**, 47–56.

Tarin, J. J. (1996) Potential effects of age-associated oxidative stress on mammalian oocytes/embryos. *Mol. Hum. Reprod.*, **2**, 717–724.

Weinberg, C.R. and Wilcox, A.J. (1995) A model for estimating the potency and survival of human gametes *in vivo*. *Biometrics*, **51**, 405–412.

Weinberg, C.R., Moledor, E., Baird, D.D. *et al.* (1994a) Is there a seasonal pattern in early pregnancy loss? *Epidemiology*, **5**, 484–489.

Weinberg, C.R., Gladen, B.C. and Wilcox, A.J. (1994b) Models relating the timing of intercourse to the probability of conception and the sex of the baby. *Biometrics*, **50**, 358–367.

Wilcox, A.J., Weinberg, C.R., Wehmann, R.E. *et al.* (1985) Measuring early pregnancy loss: laboratory and field methods. *Fertil Steril.*, **44**, 366–374.

Wilcox, A.J., Weinberg, C.R., O'Connor, J.F. *et al.* (1988) Incidence of early loss of pregnancy. *New Engl. J. Med.*, **319**, 189–194.

Wilcox, A.J., Weinberg, C.R. and Baird, D.D. (1990) Risk factors for early pregnancy loss. *Epidemiology*, **1**, 382–385.

Wilcox, A.J., Weinberg, C.R. and Baird, D.D. (1995) Timing of sexual intercourse in relation to ovulation: Effects on the probability of conception, survival of the pregnancy and sex of the baby. *New Engl. J. Med.*, **333**, 1517–1521.

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