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The radiosensitivity of the human oocyte

W.H.B.Wallace[1,3], A.B.Thomson[1] and T.W.Kelsey[2]

[1]Section of Child Life and Health, Department of Reproductive and Developmental Sciences, University of Edinburgh and [2]School of Computer Science, University of St Andrews, UK

[3]To whom correspondence should be addressed at: Department of Haematology/Oncology, Royal Hospital for Sick Children, 17 Millerfield Place, Edinburgh EH9 1LW, UK. E-mail: hamish.wallace@luht.scot.nhs.uk

BACKGROUND: We determined the best model available for natural follicle decline in healthy women and used this to calculate the radiosensitivity of the human oocyte. METHODS: Ovarian failure was diagnosed in six patients with a median age of 13.2 years (range 12.5–16.0) who were treated with total body irradiation (14.4 Gy) at 11.5 years of age (4.9–15.1). We previously estimated the dose of radiation required to destroy 50% of the oocytes (LD_{50}) to be <4 Gy. This estimate is an oversimplification, because decay represents an instantaneous rate of temporal change based upon the remaining population pool, expressed as a differential equation: $dy/dx = -y[0.0595 + 3716/(11780 + y)]$, with initial value $y(0) = 701\,200$. RESULTS: Solving the differential equation, we have estimated the number of follicles left after irradiation given as *sol*(51 – s + r), where r equals age at treatment, s equals age at diagnosis of ovarian failure, and 51 years is the average age of menopause. The surviving fraction of oocytes as a percentage is 100 times this value divided by *sol*(r). The mean surviving fraction for the six cases is 0.66%. We obtain a function, g(z), which decreases in value from 100% at zero dosage to mean value at dosage z = 14.4 Gy. We have $g(z) = 10^{mx+c}$, where $c = \log_{10}100 = 2$, and $m = [\log_{10}(0.66) - c]/14.4$. Solving $g(z) = 50$ gives an LD_{50} of 1.99. CONCLUSIONS: Based on new data and a revised mathematical model of natural oocyte decline, we have determined the surviving fraction of oocytes following irradiation and estimate the LD_{50} of the human oocyte to be <2 Gy.

Key words: fertility/human oocyte/ovarian failure/radiotherapy

Introduction

Females treated for cancer with abdominal, pelvic or total body irradiation (TBI) may sustain permanent damage to the ovary and loss of primordial follicles, resulting in impaired fecundity and a premature menopause. An understanding of the dynamics of ovarian follicle decline will facilitate our ability to predict the impact of treatment on ovarian function and to manage fertility issues in these young women more scientifically.

The human ovary is endowed with a fixed pool of primordial follicles, maximal at 5 months of gestational age, which steadily declines throughout life, culminating in the menopause at an average age of 51 years. Follicle depletion, as a result of atresia and recruitment towards ovulation, leads to premature exhaustion of the follicle pool and menopause long before death, in contrast to other mammals. A number of mathematical models have been proposed in humans to describe the rate of follicle decline based on a series of data describing the number of follicles present at different ages in humans (Block, 1952, 1953; Richardson *et al*., 1987).

For any given age, the size of the follicle pool can be estimated based upon a mathematical model of decline. The rate of oocyte decline represents an instantaneous rate of temporal change, based upon the remaining population pool. Therefore, reduction of the follicle pool as a consequence of cytotoxic therapy will result in premature exhaustion of the pool, and advance the onset of the menopause. In order to predict the age of menopause in patients who have experienced radiotherapy to a field that includes the ovaries, the extent of the radiation-induced damage to the follicle pool must be determined.

We have previously estimated the dose of radiation required to destroy 50% of primordial follicles (LD_{50}) to be <4 Gy (Wallace *et al*., 1989a). For a given dose of radiotherapy, the surviving fraction can be determined and the age of menopause predicted by applying a mathematical model for decay. In this way, patients can be counselled appropriately with regard to their reproductive life span and their window of opportunity for fertility (Wallace *et al*., 2001).

Methods

Natural decline of the ovarian follicle pool

Data estimating the number of ovarian follicles available at different ages in humans are found in a number of studies, and such data have been used to construct complex mathematical models of follicle decline (Block, 1952, 1953; Baker, 1963; Gougeon, 1984; Richardson

Table I. Age at irradiation, age at diagnosis of ovarian failure, and surviving fraction of oocytes for eight cases (Bath *et al*., 1999)

Case	Age at irradiation (years)	Age ovarian failure diagnosed (years)	Surviving fraction of oocytes (%)
1	15.1	15.6	0.40
2	12.7	13.3	0.34
3	12.5	13	0.33
4	13.6	16	0.68
5	10.5	13	0.56
6	7.9	NF	NA
7	5.9	NF	NA
8	4.9	12.5	1.68

NF = normal ovarian function; NA = not applicable.

et al., 1987). The application of such a model will enable the number of follicles present to be predicted as a mathematical product of the initial follicle store and the rate of subsequent attrition for any given age.

It is estimated that during embryo development, approximately seven million germ cells are formed in the ovarian rudiment, but only two million are present at birth and 300 000 by menarche (Block, 1952; Baker, 1963). The precise number of oocytes remaining at menopause is unclear. Ovarian follicles have been counted in the ovaries of 43 females aged 6–44 years, following accidental death, and the number of follicles present at menopause was predicted, using linear extrapolation, to be 2200 (Block, 1952). This is likely to be an overestimate, as further studies of follicle numbers present in the ovaries of pre-, peri- and post-menopausal women have demonstrated that <1000 ovarian follicles remain in peri-menopausal women, indicating that follicle decline accelerates in the decade preceding menopause (Richardson *et al*., 1987). With only an estimated 400 ovulations occurring during the reproductive period, this progressive reduction is attributable to follicle death by apoptosis.

Evidence for temporal decline has been well characterized in rodents, and early mathematical models were constructed based on a model of negative exponential rate of decay: $y = A \exp(-bx)$, where A is the number of follicles present at birth; or, following a logarithmic transformation to give a model for linear decay: $\log(y) = \log(A) - bx$, where x refers to age, y to the number of follicles and $b(>0)$ is the rate of exponential decay (Faddy *et al*., 1983).

The rate of decline (b) was then determined by applying simple regression analyses of logged number of follicles against age. Each model can be described as $y(x) = f(x)$ for x between zero (birth) and the estimated age of menopause, where $y(x)$ is the estimated number of follicles present at age x, and $f(x)$ is a function used to obtain $y(x)$. The proposed mathematical models vary depending upon the chosen subset of data describing follicle numbers at different ages. Richardson *et al*. provided the mathematical model (Richardson *et al*., 1987):

$\log_{10}(y) = 6.13 \pm 0.33 - 0.06 \pm 0.01x$
based on one source of data (Block, 1952), and:

$\log_{10}(y) = 5.94 \pm 0.37 - 0.04 \pm 0.01x$
based on another source of data (Gougeon *et al*., 1984).

We previously described the function $\log_{10}(y) = 6.3 - 0.06x$, based on two sources of data (Block, 1952; Baker, 1963).

The above models are constructed using data describing a population aged 6–44 years. Therefore, it is with some reservation that these models have a wider application from birth to menopause. Furthermore, none of the above models make use of more than two data sources, and a larger data set is likely to produce a more accurate model.

Construction of the above models is based on the assumption that ovarian follicle decline follows simple exponential decay; however, it is now recognized that the loss of ovarian follicles is more complex. A graphical representation of ovarian follicle number, expressed logarithmically against age, suggested that ovarian follicle decline is bi-exponential with 'broken-stick' regression (Faddy *et al*., 1992). An increase in the rate of exponential decline appeared to occur at age 38 years, corresponding to a follicle pool of 25 000. Following these observations, Faddy *et al*. proposed a more comprehensive model of piecewise exponential decay (Faddy *et al*., 1992) based on a least squares fit to data from three other authors (Block, 1952, 1953; Gougeon, 1984; Richardson *et al*., 1987):

$\log_e(y) = 952\,000 - 0.097x$ for $0 < x < 37.5$
and:

$\log_e(y) = e^{19.02} - 0.237x$ for $37.5 < x < 51$

Although data from Baker were not used (Baker, 1963), the value at birth of 952 000 is in line with his results, and assumes a population of 1000 follicles at a menopause occurring at age 51 years. However, this model fails to concord with the distribution of menopausal ages described by Trelour (Trelour, 1981). Furthermore, biologically, this abrupt change when the oocyte population falls to 25 000 is unlikely; more plausibly, the change is likely to represent an instantaneous rate of temporal change based on the remaining population pool, which is expressed mathematically as a differential equation. Faddy and Gosden provided a revised model (Faddy and Gosden, 1996) obtained by incorporating Trelour's data into a least squares analysis of the four quantitative studies in terms of the differential equation:

$dy/dx = -y[0.0595 + 3716/(11\,780 + y)]$ (1)
with initial value $y(0) = 701\,200$.

We consider this to be the best model currently available, and have solved it to revise our estimate of the radiosensitivity of the human oocyte based upon additional data from young women who developed ovarian failure following treatment with TBI.

Patients

We studied two cohorts of women with ovarian failure secondary to radiotherapy treatment for childhood cancer. The first cohort comprised of eight post-pubertal women, median age 17.1 years (range 15.4–21.5), recruited from paediatric oncology late effects clinics throughout Scotland (Bath *et al*., 1999) (Table I). The patients had been treated with TBI, 14.4 Gy in eight fractions over 3 days, during first or second remission for leukaemia at age 11.5 years (range 4.9–15.1). No shielding to the ovary was applied. All patients received chemotherapy with standard Medical Research Council (MRC) trials for acute lymphoblastic leukaemia or acute myeloid leukaemia.

Table II. Age at irradiation, age at diagnosis of ovarian failure, and surviving fraction of oocytes for the 19 cases studied by Wallace *et al.* (Wallace *et al*., 1989a,b)

Case	Age at irradiation (years)	Age ovarian failure diagnosed (years)	Surviving fraction of oocytes (%)
1	13	14.1	0.42
2	2	13.1	2.91
3	4	13.2	2.28
4	5	12.8	1.77
5	13	15.7	0.72
6	8	12	0.77
7	3	11.9	1.99
8	2	9.7	1.42
9	4	10.5	1.19
10	4	10.4	1.16
11	2	14.3	3.59
12	2	15.9	4.64
13	11	13.9	0.67
14	12	12.6	0.33
15	4	13.6	2.48
16	1	11.7	2.53
17	6	12	1.19
18	3	11.1	1.66
19	7	NF	NA

NF = normal ovarian function; NA = not applicable.

The second cohort comprised 19 patients from our previous study describing the radiosensitivity of the human oocyte (Wallace *et al*., 1989a) (Table II). The 19 patients had been treated during childhood for an intra-abdominal tumour with whole abdominal radiotherapy (total dose: 30 Gy, 16–26 fractions), surgery and chemotherapy between 1966 and 1975 at the Christie Hospital, UK (Wallace *et al*., 1989b). The median age at treatment was 4 years (range 1.3–13.1) and the underlying malignancies included Wilms' tumour (n = 12), unilateral ovarian dysgerminoma (n = 3), adrenal carcinoma (n = 1), sacro-coccygeal teratoma (n = 1), bladder rhabdomyosarcoma (n = 1) and abdominal neuroblastoma (n = 1). Radiotherapy was delivered using a two-field technique by either a linear accelerator or a telecobalt machine to a field defined superiorly by the dome of the diaphragm and inferiorly by the superior border of the ischial tuberosity. Shielding was applied throughout to the femoral heads and acetabula, and in the Wilms' tumour patients to the unaffected kidney after 20 Gy. Both ovaries lay within the irradiation field, and though the exact anatomical position was not defined, any shielding applied did not involve the pelvis and therefore the total dose delivered (30 Gy) was likely to have been received by both ovaries. Eight patients received no chemotherapy and the remaining 11 received chemotherapeutic agents that are not known to be gonadotoxic (vincristine, adriamycin and actinomycin D).

A full menstrual history was taken, pubertal status was assessed according to the Tanner criteria, and baseline serum FSH, LH and estradiol were measured using standard radioimmunoassays at each routine clinic visit. Ovarian failure was defined as failure to undergo or complete pubertal development, or the onset of a premature menopause before age 40 years, in association with persistently elevated gonadotrophin levels (FSH and LH >32 IU/l) and low estradiol concentration (<40 pmol/l). For patients on sex steroid replacement, spontaneous ovarian function was assessed after discontinuing treatment for a minimum of 4 weeks.

From the first cohort, ovarian failure developed in six of the eight subjects at a median age of 13.2 years (range 12.5–16.0) and all six had received sex steroid replacement therapy (Table I). The remaining two patients had progressed spontaneously through puberty without sex steroid replacement therapy, although they had irregular menstrual cycles and intermittently elevated gonadotrophins. In the second cohort, premature ovarian failure occurred in 18 of the 19 women, at a median age of 12.7 years (range 9.7–15.9; Table II).

We have obtained a solution to the differential equation described by Faddy and Gosden above (Faddy and Gosden, 1996) using a seventh-eighth order continuous Runge–Kutta numerical method. Application of the Faddy–Gosden model for healthy untreated women aged 0–51 years enables the oocyte population to be determined for any given age (Figure 1).

Results

Using the numerical solution procedure, *sol*, derived as above, we obtained a procedure which takes an age at irradiation and age of diagnosis of ovarian failure, and returns (as a percentage) a surviving fraction of oocytes. Suppose child A is treated at age r, and has ovarian failure diagnosed at age s. Then, assuming that (i) decay occurs after irradiation at the rate given by the Faddy–Gosden model, and (ii) age of ovarian failure is close to the age of diagnosis of ovarian failure, we can estimate that the number of follicles remaining after irradiation is $sol(51 - s + r)$. The surviving fraction as a percentage is then 100 times this number divided by $sol(r)$. The mean surviving fraction for the six cases in the first cohort is 0.66% (see Table I). If we assume a logarithmic increase in the number of follicles removed with increasing dose, then we can obtain a function, g(z), which decreases in value from 100% at zero dosage to the mean value at dosage z = 14.4 Gy given to the six cases. We have $g(z) = 10^{mx+c}$, where $c = \log_{10}100 = 2$, and $m = [\log_{10}(0.66) - c]/14.4$.

Solving g(z) = 50 gives an LD_{50} of 1.99 Gy

Figure 2 demonstrates the estimation of LD_{50} for the human oocyte. The dose required to completely destroy the follicle pool, D_0 (LD_{100}), is an infinite number of Gy, since we are assuming a logarithmic model.

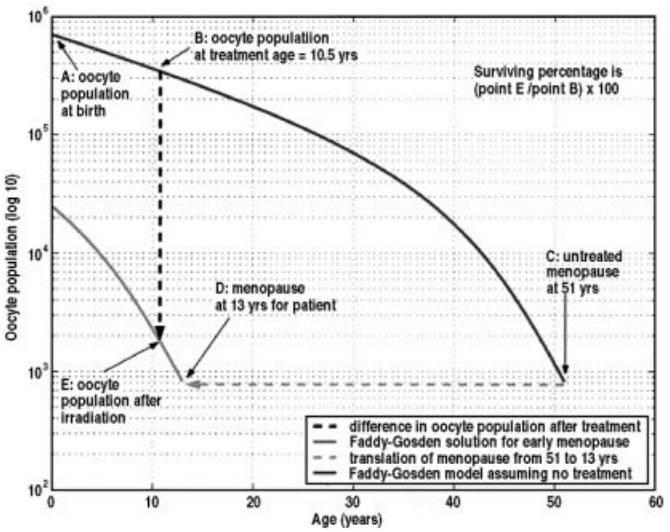

Figure 1. The Faddy–Gosden model. The solution of the Faddy–Gosden equation enables the size of the oocyte pool to be determined for any given age from birth to menopause, at an estimated age of 51 years. The calculation of an estimated surviving fraction for case 5 in Table I is shown: ovarian failure occurred at age 13 years in case 5. By applying the Faddy–Gosden model, we can determine the size of the surviving fraction following radiotherapy at age 10.5 years to be 0.56%.

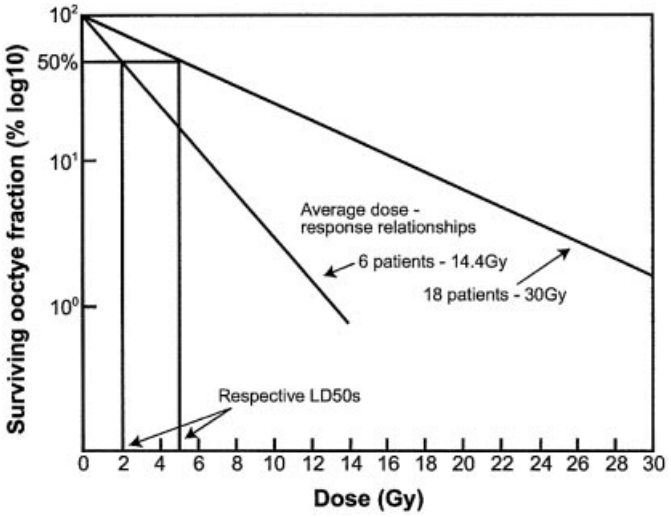

Figure 2. Dose–response relationships for the human oocyte. The mean surviving fraction of oocytes for each patient has been calculated and plotted against the dose of radiation received: (i) for the six patients who received 14.4 Gy, and (ii) for the 18 patients who received 30 Gy. These lines represent the estimated (fractionated) dose–response relationship for the human oocyte. The LD_{50} is given by the dose required to leave a surviving fraction of 50%. Our revised LD_{50} of <2 Gy is taken from the relationship obtained by data from the cohort of six patients.

We previously determined the radiosensitivity of the human oocyte based on an analysis of the 19 patients treated with whole abdominal irradiation. The surviving fraction of oocytes following irradiation was calculated for each of the 18 patients who developed ovarian failure. By plotting the surviving fraction logarithmically against the dose of radiation received, the LD_{50} was estimated to be <4 Gy. With new evidence (Faddy *et al*., 1992), it is clear that primordial follicle decay does not follow a simple logarithmic decline, but rather represents an instantaneous rate of temporal change based upon the remaining population pool. Using the new mathematical model of follicle decline, we determined the surviving oocyte fraction in our second population of 18 patients with ovarian failure and revised our estimate of the LD_{50} for this group. Replacing z with a dose of 30 Gy for 14.4 Gy for the second cohort, our original data set of 18 cases (Table II) gives an LD_{50} of 5.15 Gy.

Discussion

We estimate the LD_{50} of the human oocyte to be <2 Gy, calculated by applying the solution of the Faddy–Gosden mathematical model of ovarian follicle decay to six patients with ovarian failure secondary to TBI as part of the treatment for childhood cancer. The human ovary is endowed with a fixed number of primordial follicles at birth, which undergo attrition and recruitment towards ovulation, culminating in menopause. Ovarian failure and a premature menopause will ensue from any cytotoxic insult, which either depletes the oocyte pool or hastens its decline. Radiotherapy is well recognized to cause destruction of the ovarian pool, although the extent of the damage is difficult to determine. Ovarian failure following radiotherapy during childhood may manifest in adolescence as failure of spontaneous puberty or pubertal arrest, or in adulthood as infertility and premature menopause.

The aetiology of ovarian failure in both our cohorts of patients is likely to be irradiation induced. The patients in the first cohort who had received TBI were treated with standard MRC chemotherapy protocols that include alkylating agents and cytosine. Ovarian function appeared to be preserved after standard treatment for acute lymphoblastic leukaemia (Wallace *et al*., 1993). We have described decreased LH secretion and short luteal phases in young women whose treatment included low dose cranial irradiation (Bath *et al*., 2001), but premature ovarian failure is not described, although these women may go on to have an early menopause.

With no biochemical markers available to predict those patients for whom premature ovarian failure is likely, information to determine the extent of radiotherapy-induced damage and prediction of the likely fertile window will be helpful for reproductive counselling. Oocyte radiosensitivity differs tremendously between species, with the mouse oocyte (LD_{50} 0.15 Gy) being about 350 times more radiosensitive than that of the monkey (LD_{50} 50 Gy). The radiosensitivity of the human oocyte has been reported in a number of studies, although the majority of these have focused on radiotherapy treatment in adult patients. Bianchi estimated the LD_{50} for human oocytes to be 6–18 Gy (Bianchi, 1983). In these patients, radiotherapy was administered for the treatment of benign gynaecological disorders to induce an artificial menopause. Permanent ovarian failure was induced in a group of 72 patients, most of whom were >40 years old at time of treatment, following administration of 625 roentgens (~6 Gy)

(Bianchi, 1983). In a study of 2000 females treated with radiotherapy for menorrhagia, permanent ovarian failure was induced in 97% of patients following irradiation with 5–10.5 Gy. In this study, our revised estimate of the LD_{50} for the human oocyte is significantly less than previously reported.

We have based our calculations on the assumption that the rate of decline of the surviving fraction of oocytes is not greater than that for a non-irradiated ovary. This is based upon the observation that oocytes die in interphase within a few hours of irradiation, becoming pyknotic and then removed by phagocytosis within a few days (Lindop, 1969). Furthermore, in irradiated fetal rat ovaries, severely damaged germ cells degenerate rapidly and are eliminated from the ovary within a few days of exposure. It is reported that the subsequent rate of oocyte depletion is lower in irradiated animals than in controls (Beaumont, 1964). If this were true, then the surviving fraction for each patient would be lower than we have estimated, indicative of increased radiosensitivity of the human oocyte and, consequently, a lower LD_{50}.

Radiotherapy treatment before puberty may result in significant ovarian follicle depletion. However, the earliest clinical manifestation of ovarian follicle exhaustion is failure of pubertal development in association with elevated gonadotrophins. Delay in the diagnosis of ovarian failure would result in an overestimation of the LD_{50}. Estimation of the LD_{50} (<2 Gy) for the cohort of patients treated with 14.4 Gy TBI was lower than our previous estimate of that (4 Gy) for our original cohort of 19 patients who received 30 Gy abdominal irradiation. The LD_{50} of 4 Gy is likely to be an overestimation, largely attributable to the long lag period between treatment and the earliest detection of clinical ovarian failure. It is now clear that the oocyte pool of these females would have been exhausted at an earlier age than that at which clinical manifestations could be detected. Using the solution to the Faddy–Gosden model has enabled us to recalculate the LD_{50} for the original cohort and, to our surprise, the estimate (5.15 Gy) is higher than we previously reported. The reason for the overestimate remains the higher radiation dose received by the first cohort, who were treated at a younger age, with a resulting longer lag period between treatment and the detection of ovarian failure. We therefore maintain that the most accurate estimate (LD_{50} <2 Gy) is calculated from applying the Faddy–Gosden solution to the second cohort, who were treated at an older age [median 11.5 (range 4.9–15.1) versus 4 (1–13) years] and with a lower total radiation dose.

A further consideration when applying our construct clinically is the uncertainty of the impact of fractionated doses of radiotherapy. Our estimation of the LD_{50} may be considered as an upper limit, because it does not take into consideration the fractionated schedule of radiotherapy.

Solving the Faddy–Gosden mathematical model for ovarian follicle decline using a seventh-eighth order continuous Runge–Kutta numerical method, and applying the solution to new clinical data on age at development of ovarian failure after TBI (14.4 Gy), has enabled a more accurate estimate of the radiosensitivity of the human oocyte. Calculation of the dose of radiation received by each ovary, combined with a more accurate estimate of the radiosensitivity of the human oocyte, will facilitate our ability to provide more scientific fertility counselling to young women at risk of a premature menopause following the successful treatment of cancer.

References

Bath, L.E., Critchley, H.O., Chambers, S.E., Anderson, R.A., Kelnar, C.J. and Wallace, W.H.B. (1999) Ovarian and uterine characteristics after total body irradiation in childhood and adolescence: response to sex steroid replacement. *Br. J. Obstet. Gynecol.*, **106**, 1265–1272.

Bath, L.E., Anderson, R.A., Critchley, H.O., Kelnar, C.J. and Wallace, W.H. (2001) Hypothalamic–pituitary–ovarian dysfunction after prepubertal chemotherapy and cranial irradiation for acute leukaemia. *Hum. Reprod.*, **16**, 1838–1844.

Baker, T. (1963) A quantitative and cytological study of germ cells in human ovaries. *Proc. R. Soc. (Biol.)*, **158**, 417–433.

Beaumont, H.M. (1964) The short-term effects of acute X-radiation on oogonia and oocytes. *Proc. R. Soc*., **161**, 550–570.

Bianchi, M. (1983) Cytotoxic insult to germinal tissue, part 2; The ovary. In Potten, C.S. and Hendry, J.H. (eds) *Cytotoxic Insult to Tissue: Effects on Cell Lineages*. Churchill-Livingstone, Edinburgh, UK, pp. 309–328.

Block, E. (1952) Quantitative morphological investigations of the follicular system in women: variations at different ages. *Acta Anat.*, **14**, 108–123.

Block, E. (1953) A quantitative morphological investigations of the follicular system in newborn female infants. *Acta Anat.*, **17**, 201–206.

Faddy, M.J. and Gosden, R.G. (1996) A model conforming the decline in follicle numbers to the age of menopause in women. *Hum. Reprod.*, **11**, 1484–1486.

Faddy, M.J., Gosden, R.G. and Edwards, R. (1983) Ovarian follicle dynamics in mice: a comparative study of three inbred strains and an f1 hybrid. *J. Endocrinol.*, **96**, 23–33.

Faddy, M.J., Gosden, R.G., Gougeon, A., Richardson, S.J. and Nelson, J.F. (1992) Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum. Reprod*, **7**, 1342–1346.

Fauser, B.C. and van Heusden, A.M. (1997) Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr. Rev.*, **18**, 71–106.

Gosden, R.G. (1987) Follicular status at the menopause. *Hum. Reprod.*, **2**, 617–621.

Gougeon, A. (1984) Caractéres qualitatifs et quantitatifs de la population filliculaire dans l'ovaire humain adulte. *Contracep. Fertil. Sex.*, **12**, 527.

Lindop, P.J. (1969) The effects of radiation on rodent and human ovaries. *Proc. R. Soc. Med.*, **62**, 144–148.

Richardson, S.J., Senikas, V. and Nelson, J.F. (1987) Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *Clin. Endocrinol. Metab*., **65**, 1231–1237.

Trelour, A. (1981) Menstrual cyclicity and the pre-menopause. *Maturitas*, **3**, 249–264.

Wallace, W.H.B., Shalet, S.M., Hendry, J.H. *et al.* (1989a) Ovarian failure following abdominal irradiation in childhood: the radiosensitivity of the human oocyte. *Br. J. Radiol.*, **62**, 995–998.

Wallace, W.H.B., Shalet, S.M., Crowne, E.C. *et al.* (1989b). Ovarian failure following abdominal irradiation in childhood: Natural history and prognosis. *Clin. Oncol.*, **1**, 75–79.

Wallace, W.H.B., Shalet, S.M., Tetlow, L.J. and Moris-Jones, P.H. (1993) Ovarian function following the treatment of childhood acute lymphoblastic leukaemia. *Med. Pediatr. Oncol.*, **21**, 333–339.

Wallace, W.H.B., Blacklay, A., Eiser, C. *et al.* (2001). Developing strategies for long-term follow up of survivors of childhood cancer. *Br. Med. J.*, **323**, 271–274.

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