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Does a period of amenorrhoea raise subsequent chances of implantation in women?

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A notable feature of the recent work on IVF has been the use of oocyte donation and hormone replacement therapy (HRT) to establish pregnancies in women. This form of treatment originated through the work of Lutjen *et al.*[1], when the first pregnancy arose through the use of a sequence therapy with oestrogens and progestagens to imitate the steroidal events of the natural menstrual cycle in combination with oocyte donation and embryo transfer. Since then, several studies have shown how this form of treatment in acyclic women, e.g. those between the ages of 30 and 50, can result in pregnancy rates far higher than in cyclic women of the same age undergoing IVF with their own oocytes[2]. The lengthy debate on the causes of this higher fertility, and especially the respective roles of egg and uterus, has not led to any clear resolution of this condition. Some workers indicated that the uterus is the primary factor leading to high rates of implantation[2, 3], whereas others insisted on the donation of oocytes from younger women as the primary factor[4]. The concepts underlying these debates have even led to some clinics recommending premenopausal women to have oocytes from donors instead of using their own oocytes to establish pregnancies, to take advantage of the higher rates of implantation supposedly gained by this approach.

This brief review gives our own concepts on the causes of high fecundity in acyclic women, and extends these concepts to the establishment of high rates of pregnancy in women who are over 50 years, or are down-regulated for several months by the use of LHRH agonists. We also propose reasons for the high fecundity of young women, and an explanation of the sharp decline in fertility of women approaching menopause.

Is the reduced fecundity in premenopausal women due to a failure of the first stage of implantation?

The onset of infertility in premenopausal women may be due to a sudden loss in their ability to implant their embryos, i.e. during the first or a very early stage of the implantation process. Moreover, a period of amenorrhoea may be beneficial for the establishment of pregnancy in these and other women. This concept emerged from the studies on acyclic/agonadal women aged between 30 and 50 years, when a detailed analysis showed how the overall incidence of clinical pregnancies and implantation rates per embryo were significantly higher in them after accepting oocyte donation and HRT therapy than in cyclic women of similar ages[2] (Tables 1 and 2). It was shown that embryo quality, as assessed morphologically, was not the cause of this difference. Such evidence implied that no benefit arose from the use of oocytes from donors aged 35 and less.

When data from all cyclic and acyclic women were analysed, it was evident that the implantation rate per embryo was much higher in acyclic women. When data from *pregnant* women only were analysed, this difference was lost, for rates of implantation were identical in pregnant women in both the cyclic and acyclic groups (Table 2). This evidence inferred that uterine capacity in

Table 1. Pregnancy rates by age and types of cycle[2]

	Type of cycle		
UK data	Natural	HRT cyclic	HRT acyclic
Late 20s	6/18 (0 33)	9/26 (0 33)	1/3 (0 33)
Early 30s	25/77 (0 33)	15/50 (0 30)	7/12 (0 58)
Late 30s	25/77 (0 33)	17/59 (0.29)	5/8 (0 63)
> 40	1/20 (0 05)	2/20 (0 10)	1/4 (0 25)
US data			
	Stimulated		HRT acyclic
Late 20s	14/40 (0.35)		2/7 (0 26)
Early 30s	16/61 (0 26)		5/7 (0 71)
Late 30s	14/58 (0 24)		7/19 (0 37)
> 40	1/36 (0 03)		5/18 (0 28)

Table 2. Implantation rates per embryo in cyclic women and in acyclic women given oocyte donation and HRT therapy[2]

Cycle	All patients	Pregnant patients only
UK data		
Natural	68/482 (0 141)	68/165 (0 412)
HRT cyclic	55/387 (0 142)	55/131 (0 420)
HRT acyclic	16/60 (0 267)	16/36 (0.444)
US data		
Stimulated	57/549 (0 104)	57/176 (0 324)
HRT acyclic	19/120 (0.158)	19/48 (0 396)

fertile women of both the groups was similar, and that the fertility difference between them arose because significantly more acyclic than cyclic women could establish pregnancies.

These findings indicate that the decline in fertility of cyclic women as menopause approaches is due to a failure of implantation in an increasing proportion of them. It is not due to a general decline in the fertility of all women. Moreover, the onset of this loss of fertility is sudden, and it apparently affects the first stage of implantation since there is no evidence of increasing rates of very early pregnancy loss in these premenopausal women, e g. of a high frequency of biochemical pregnancies. The women simply and suddenly fail to become pregnant, as if the embryo has either failed to attach to the uterine epithelium or has died before it could produce HCG[5]. All this evidence points to their sudden infertility being due to a catastrophic failure in the initial stages of implantation. This situation does not arise in acyclic women, who retain their youthful fertility, probably because their uterus has been rested over a period of amenorrhoea.

The significance of amenorrhoea as a means of raising implantation rates

A study was obviously needed to discover if a period of induced amenorrhoea in cyclic women could raise their chances of pregnancy when the treatment ended and their normal cycles resumed. It is not easy to find a group of infertile women who could be asked ethically to volunteer for a prolonged period of amenorrhoea before receiving IVF. They would have to forego their chances of pregnancy for several months, at a time when their desire was to establish pregnancy as soon as possible. One group of patients who could benefit from a period of amenorrhoea were those with severe endometriosis, for pituitary down-regulation would remove any stimulus to the regrowth of their endometriosis while any effects on their subsequent fertility were assessed.

A number of volunteers who had been fully counselled and who had been treated for severe endometriosis with Danazol or other treatments before undertaking IVF joined the trial. A group of control patients were given their IVF when their therapy for endometriosis had been completed. The treated patients were down-regulated using the LHRH agonist Zoladex for between 2–7 cycles before their IVF began, and results from the two groups were compared (Tables 3 and 4)[6]. Considerable differences in the resulting fertility of the two groups during IVF were found, following a minimum of 3 cycles of down-regulation. Pregnancy rates more than doubled above controls by this treatment. The degree of persistence of endometriotic lesions was similar in treated and control patients, and did not seem to influence the results.

Table 3. Outcome of treatment in successive IVF cycles in controls and following a period of pituitary down-regulation using Zoladex[6]

	Controls						Zoladex for 2–7 cycles					
Cycle no.	Number of cycles	Abandoned cycles	Freeze all embryos	Failure of fertil-ization	Number of embryo transfers	Clinical pregnancies (%)	Number of cycles	Abandoned cycles	Freeze all embryos	Failure of fertil-ization	Number of embryo transfers	Clinical pregnancies (%)
1	69	6	5	4	54	8 (14 8)	35	1	1	3	30	12 (40)
2	42	1	4	2	35	6 (17.1)	10	–	–	–	10	6 (60)
3	21	1	–	–	20	2 (10 0)	2	–	–	–	2	0 (0)
3+	26	1	–	–	25	1 (4 0)	–	–	–	–	–	–
Total	158	9	9	6	134	17 (12.7)	47	1	1	3	42	18 (42 9)

Table 4. Pregnancy rate in controls and in patients following a period of pituitary down-regulation using Zoladex in women of different ages[6]

	Controls						Down-regulated					
Age group	Number of cycles	Abandoned cycles	Freeze all embryos	Failure of fertil-ization	Number of embryo transfers	Clinical pregnancies (%)	Number of cycles	Abandoned cycles	Freeze all embryos	Failure of fertil-ization	Number of embryo transfers	Clinical pregnancies (%)
20–29	34	2	2	–	30	5 (16 6)	17	–	–	1	16	7 (43 8)
30–34	42	4	3	2	33	4 (12.1)	19	–	–	1	18	9 (50 0)
35–39	65	2	3	3	57	6 (10.5)	10	–	1	1	8	2 (25 0)
39+	17	1	1	1	14	2 (14 3)	1	1	–	–	–	–
Total	158	9	9	6	134	17 (12.7)	47	1	1	3	42	18 (42 9)

These data enable two conclusions to be drawn. First, down-regulation for 3 months or more enables the uterus to recover and high numbers of pregnancies to be established (Table 5). Secondly, this result is achieved using the patients' own oocytes, showing that oocyte donation is not needed to improve the chances of a woman implanting her own embryos. Another development in the technique of oocyte donation occurred at this time, for several clinics showed how postmenopausal women up to and beyond the age of 60 could establish pregnancy using HRT and oocyte donation.

Pregnancies in women aged over 50 years using HRT therapy and oocyte donation

Initial studies on establishing pregnancies in postmenopausal women using HRT therapy with oocyte donation were carried out in 1988 (ref. 4). The age limit in this initial study was < 50; nevertheless, it was clear that the onset of menopause did not prevent many pregnancies being established in these women. More reports[7–10] then appeared, showing how patients in the early and late 50s, and even older, could establish pregnancies in this manner (Table 6).

The number of treatment cycles with these patients is still modest, but sufficient evidence has accumulated to reach a preliminary estimate of fertility of women aged over 50. Surprisingly, their fertility was quite high, with implantation rates similar to women in their early 30s, low abortion rates and a capacity to carry their pregnancies to full term. These results were assessed in data collected from four clinics practising the work (Table 6). Some of the reported pregnancy rates were very high, e.g. 38% per attempt and 19% per embryo[10], with reports of a 29% pregnancy rate elsewhere[8]. There was overwhelming evidence that these women did not share the increasing risk of infertility of premenopausal cyclic women. Their fertility appeared to be similar to that described above of acyclic women given HRT therapy and oocyte donation, and of cyclic women given a period of down-regulation before IVF with their own oocytes. The common thread appears to be a previous period of amenorrhoea before the establishment of pregnancy is attempted.

It is also worth noting that the high fertility of young women also follows a period of amenorrhoea during their pubertal years. Perhaps this is a fourth example of the same phenomenon.

What is the significance of a period of amenorrhoea?

The most obvious value of amenorrhoea is to reduce the impact of constant cyclic steroid action on the responding uterus. If this is accepted, then there are many possible candidates to account for the beneficial effects

Table 5. Pregnancy rates related to the number of months of down-regulation and activity of endometriosis[6]

Zoladex cycles	Endometriosis after Zoladex treatment		
	Active	Non-active	Total
2	0/3	1/4	1/7
3	6/17	2/7	8/24
4–5	1/2	2/2	3/4
6–7	3/5	3/4	6/9
All 4–7	4/7	5/6	9/13

Table 6. Data from four clinics on pregnancies in women aged over 45 years

	Clinic			
	1	2	3	4
Patients	75			113
Cycles	111	114		
Failed fertilization		6		
No of embryos transferred	110	108	221	
No. of pregnancies	31	50	66	44
Pregnancy rate per embryo transfer	28 1		28 5	38 4
Implantation rate per embryo	14 4	17 4	11 9	12 6
Abortions	4	8		7

Data still incomplete for some parameters

on implantation rates. Changes in the characteristics of steroid-sensitive uterine flora, the down-regulation of steroid receptors, or perhaps an overgrowth of some tissues after repeated menstrual cycles could all be involved. The list can be narrowed down by the evidence described above that the first or an early stage of implantation is involved. Taking this into account, the accumulating effects of repeated cycles could be mediated by changes in the epithelial receptors responsible for embryo attachment (i.e. in adhesion), or perhaps those assisting the very earliest changes in the invasion of the embryo into the stroma. The most likely candidate, however, is the pinopod.

Pinopods, the 'drinking pods', are highly progesterone-sensitive, with perhaps the highest concentration of progesterone receptors in the uterus. They are thought to be responsible for extracting uterine fluid[11–13]. Removal of the fluid draws the walls of the uterus tightly around the embryo, so tight that indentations are made in the trophoblast of the embryo, in patterns resembling those of the pinopods and microvilli on the luminal epithelial surface. This tight binding might then enable the embryo to attach to this epithelium by short-range chemical forces. During the natural cycle, pinopods appear on the luminal epithelium on cycle day 18–19, and persist for 3–4 days; none remain by day 22 (ref. 14, 15). Fewer patients may form pinopods, and they may also form 1–2 days earlier after ovarian hyperstimulation, during HRT cycles or after uterine infection[16]. In HRT cycles, pinopods form by day 19, and persist to day 21, although their appearance may be postponed by two days[15]. The timing of their appearance matches the period of implantation very closely, i.e. it seems to indicate the period of the 'implantation window'.

Pinopods are just the type of organelle which would be responsible for the first stage of implantation. Their malfunction would result in the embryo remaining in a pool of uterine fluid, with little chance of binding to the luminal epithelium. This would explain the situation in women approaching menopause. There has been evidence of 'secretory exhaustion' in the uterus[17], and of a declining number of pinopods in the ageing mouse uterus[18]. 'Pinopod disease' under these circumstances could explain all the observations made on the fertility of premenopausal women, and its reversal could explain the observations described above on acyclic, postmenopausal and pubertal women, and on these cyclic women given pituitary down-regulation for several months before IVF. Could this disease be alleviated by a period of amenorrhoea?

The effects of amenorrhoea on fertility

It is unfortunate that there is very little direct evidence on the effect of a period of amenorrhoea on the functions of the uterus at implantation. Indeed, it seems that the question has never been seriously raised before, in any mammalian species. There is no evidence of the pinopod population in the human uterus at menarche, during the reproductive years and at menopause, despite the thousands of endometrial biopsies taken over so many years! There is evidence of their high progesterone sensitivity, reflected in their sensitivity to the action of antiprogestagens[19]. Despite some evidence of a decreasing number of pinopods in ageing mice[18], there is none on their changing numbers or sensitivity with advancing age in women, except for statements by some observers pointing out how they represent an excellent marker of fertility in the human female.

It is clear that the uterus can respond very quickly to steroid hormones after a period of amenorrhoea. This has been shown by the capacity of acyclic women to be capable of implanting an embryo in the first cycle of HRT therapy after many years of postmenopausal amenorrhoea[2]. There is no need for a preparative cycle, for implantation rates are similar without it. Some evidence in rodents has indicated that the chances of implantation are higher when ovariectomy is used to induce an amenorrhoeic period, succeeded by ovarian grafts[20]. It is even possible that the embryo is involved in this scenario, since the human blastocyst produces progesterone in addition to HCG[21], and may release GnRH as in the rhesus monkey blastocyst[22], and this and other embryonic hormones could exert considerable local effects on the luminal epithelium. A considerable amount of work is clearly needed, involving carefully timed endometrial biopsies coinciding with pinopod formation and regression, and detailed measurements of pinopod activity at various maternal ages. A thoughtful analysis of other potential steroid-sensitive systems which affect the first stage of implantation and which could be impaired by repeated cyclic activity is also needed.

Explanations will have to be made of the 'uterine memory' that is inherent in this model of a pinopod disease as a cause of human infertility. Which uterine factor leads to a succession of cycles that enables epithelium or stroma to 'remember' their previous exposure to steroids? This question is complicated by the nature of menstruation and the repair of the stroma from a population of stem cells. These cells must have an imprinted system responsive to steroids that is passed on to their descendant cells during successive menstrual cycles, yet are reversible by a period of amenorrhoea and steroid withdrawal. This factor is unlikely to be genetic, but it could involve shorter-term modifications in secondary messenger systems.

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Stress and reproduction: The role of peptides and other chemical messengers in the brain

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Stress is a powerful regulator of reproduction. Reproduction is reduced in both humans and animals exposed to either physical or psychological stressors. Both sexual behaviour and fertility are diminished. Recent studies suggest that a variety of neurochemical systems in the brain are involved. These include peptides, such as β-endorphin and CRF, as well as steroids and monoamines. These have powerful suppressant actions on reproduction. Though peptides are distributed within the limbic system, the precise actions they have on behaviour and other dependent variables depend, for example, upon the site at which they act. The effects of β-endorphin in the hypothalamus and amygdala, though related, are distinct. It is becoming apparent that specific peptide-containing neural systems are activated by different categories of stressor. This knowledge will lead to more precise understanding of stress-induced infertility.

Studying reproduction: strategies

The experimental study of reproduction has been highly successful. The use of standardized preparations under carefully controlled conditions has enabled the mechanisms underlying such important reproductive events as ovulation, implantation, seasonal breeding and sexual behaviour to be described and, to a degree, understood. Though no-one would argue with the premise that much more needs to be done, nevertheless current information is such that not only are there coherent accounts of these processes, but the applications of this knowledge to the clinic and the community have had immense impact on human affairs. The power of the simplifying approach to these questions is, therefore, vindicated. Reproductive events, studied in comparative isolation, have yielded to the powerful experimental techniques currently available. One extreme example of this approach is the fact that fertilization and its mechanisms can now be studied *in vitro*, allowing the contemporary methods of molecular and cell biology to be applied with exciting and fruitful results.

Reproduction in the real world

But this reductionist approach, though successful in its own terms, has carried a penalty. Reproduction takes place in the real world, a world in which many other events occur, and is subject to the environmental and social constraints typical of any other activity. Of course, ecologists and population biologists have long recognized this, and the study of such factors occupies a major part of these fields of enquiry. All mammals live in a competitive and hostile environment The struggle