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Excessive Follicular Recruitment and Growth in Mothers of Spontaneous Dizygotic Twins

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Abstract. We wished to establish the frequency, regularity and laterality of multiple ovulation in mothers of dizygotic (DZ) twins and controls. Subjects had regular menses and were not using oral contraceptives. Ovarian ultrasound scans were taken over a number of cycles in 21 mothers of DZ twins and 18 controls (including 13 mothers of monozygotic [MZ] twins). Multiple large follicles ($\geqslant$ 12 mm diameter) were seen significantly more frequently in mothers of DZ twins (13/21 mothers, 24/77 cycles, average $\pm$ SE follicles/cycle 1.34 ± 0.11) than controls (2/18 mothers, 3/31 cycles, average 1.10 ± 0.08). Both ipsilateral and contralateral multiple follicles were observed. In one case a mother of DZ twins showed multiple large follicles in 7 out of 10 cycles in which she was scanned, including both ipsi-and contralateral patterns of occurrence.

Key words: Dizygotic twinning, Multiple ovulation, Follicular development, Ovarian ultrasound

INTRODUCTION

There appear to be important genetic influences on the tendency to DZ twinning. DZ twins often seem to run in families, although the pattern of inheritance is far from clear [18,19]. There are striking racial differences; in Europeans, DZ twins are born in about 8/1000 confinements but in Africans the rate is double this or higher and in Orientals less than half. There are also maternal effects, since DZ twinning increases with maternal age and parity [2,10].

In his book "The Biology of Twinning in Man", Bulmer [2] reviewed the evidence available at that time and concluded that (a) there is no evidence for a paternal effect in DZ twinning; (b) there is a fourfold increase in frequency of DZ twins amongst the

siblings of DZ twins; (c) mothers and daughters of women who have had DZ twins are 1.8 times more likely than average to have DZ twins themselves; (d) sisters of women who have had DZ twins are 2.6 times more likely to have DZ twins themselves. From these figures Bulmer calculated that the correlation between sisters in the tendency to DZ twinning is about 0.5, and between mothers and daughters is about 0.3. These figures are consistent with a high heritability of the trait and a high degree of genetic dominance. This is what one would expect for a "fitness trait" which has been subject to intense natural selection for higher or lower levels of twinning during the evolution of mankind [15,19,8].

Bulmer [2] hypothesised that the family data are consistent with the existence of a recessive gene *t* which has a frequency about 0.5 and which in the homozygous state increases the risk of DZ twinning. These women would comprise about one quarter of the population and have a twinning rate about 32 per 1000 – ie, they would account for all the DZ births. Further evidence of the recessive nature of the inheritance of DZ twinning is provided by the fact that women of mixed racial origin have a DZ twinning rate closer to the race with low DZ twinning rate [10]. It follows from this hypothesis that only a small proportion of women genetically predisposed actually have DZ twins. Approximately 1% European women have DZ twins; if 25% are genetically predisposed to do so then the average penetrance is about 0.04. A further corollary is that 24% women will be *tt* but not have DZ twins. Possible reasons for this lack of penetrance are that they never get pregnant, because multiple ovulation only occurs in some cycles, or because embryos in excess of one are lost.

Thus, if we wish to investigate the inheritance of DZ twinning it would be helpful to know how frequently multiple ovulation occurs in mothers of DZ twins and in women not obviously so predisposed. There are no systematically gathered data in the literature on this point, and in this study we use ovarian ultrasound to examine folliculogenesis in mothers of DZ twins and controls. Scans on the same woman over several cycles give some indication of whether multiple ovulation is a regular or sporadic phenomenon.

MATERIALS AND METHODS

Mothers of DZ twins and control subjects attended for ovarian ultrasound in the late follicular phase over several successive cycles. Inclusion criteria were (1) all pregnancies had to have been achieved without hormonal induction, (2) women had to be having regular, ovulatory menstrual cycles, (3) not pregnant or lactating and (4) not using oral contraceptives or other hormone preparations. Volunteers for this study were obtained through the Australian Multiple Birth Association and affiliated mothers-of-twins clubs in SE Queensland and Sydney. Mothers of MZ twins were used as controls. Since MZ twinning is apparently a random event, quite unrelated etiologically to DZ twinning [2], these women should be typical of the population of women who are not obviously predisposed to multiple ovulation. In the control group we also included mothers of singletons for whom ultrasound scans were taken in a single cycle. After elimination of subjects from whom no useful ultrasound data could be obtained (on account of obesity, wrong timing etc), usable data were obtained from 21 mothers of DZ twins, 13 mothers of MZ twins and five other controls. All subjects were of European extraction.

It is crucial, however, to establish the correct zygosity of twins so that subjects can be correctly assigned as mothers of DZ or of MZ twins. Nine mothers had at least one pair of opposite sex (and therefore DZ) twins. Eleven mothers had pairs of same-sex twins who were judged to be DZ on account of striking differences in eye colour, hair colour or other physical features such that teachers, friends etc. had no trouble telling them apart. Another eleven mothers had twins who were identical in appearance and were frequently mistaken by close relatives and these were judged MZ. Given this information, it is well established that the reliability of zygosity assignment judged against genotyping is very high [12]. However, there were three mothers the zygosity of whose twins could not be assigned by these criteria. For two pairs we had extensive blood typing performed and on the basis of identity for all systems they were judged MZ. DNA fingerprinting [4] was used for the remaining pair who were shown to be DZ. Among the 21 mothers of DZ twins, one had three sets of twins and four had two twin pregnancies. Of the latter, one woman had produced one set of DZ and one set of MZ twins, this being confirmed by DNA fingerprinting.

Our subjects were scattered widely in South East Queensland and the Sydney area and we obtained cooperation from gynaecologists with ultrasound facilities in Brisbane, Sydney and several regional centres. Mothers attended their clinics on approximately cycle day 12, although this varied a little depending on usual cycle length, whether this fell on a weekend etc.. Ideally, all subjects would have been scanned at frequent intervals for the last few days of the follicular phase to see exactly how many follicles proceeded to ovulation. In practice, it was often difficult to arrange even one appointment. In some cases, if follicles were too small when the subject was examined it was possible to arrange for her to come back for another scan a day or two later, but in most cases practical considerations restricted us to only a single scan per cycle. Mothers of twins were encouraged to have scans for as many cycles as they (and the ultrasonographer) were willing. For one subject (no. 10) we obtained scans for ten out of twelve consecutive cycles and several others presented for scans for four or five consecutive months.

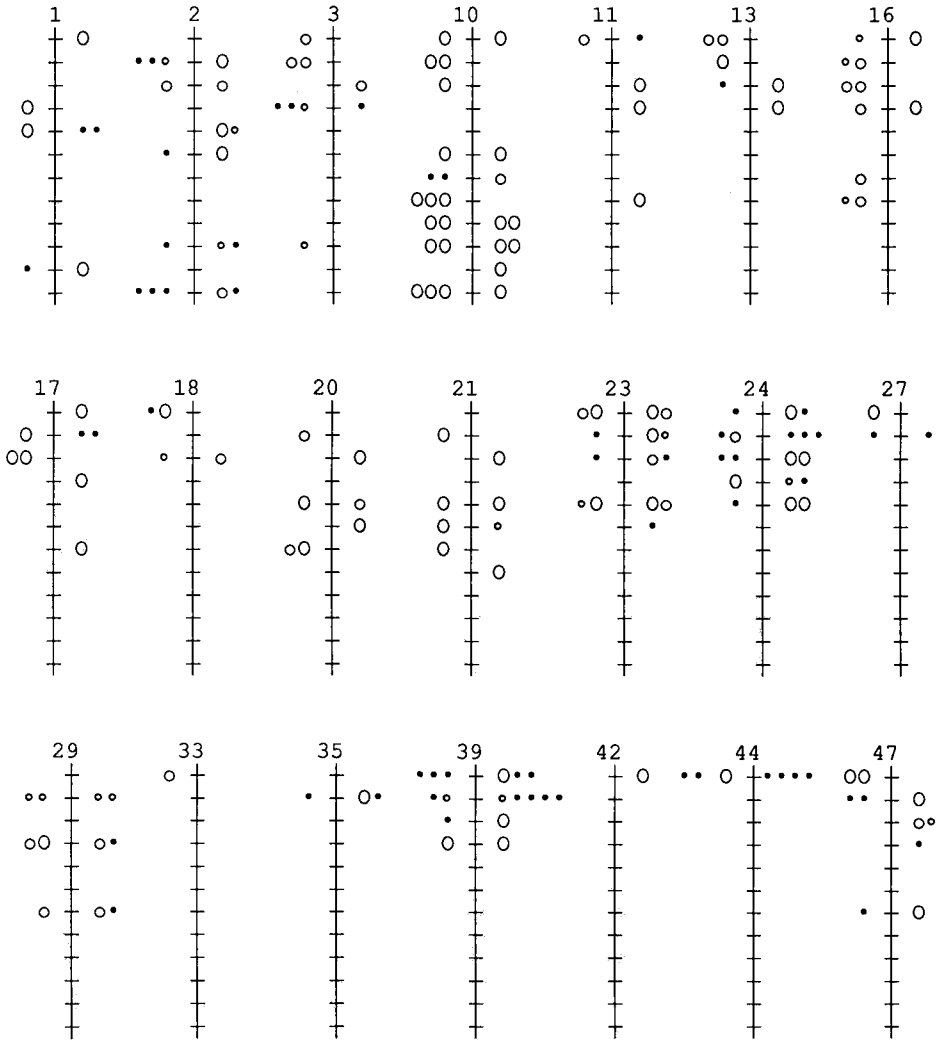
Most scans were obtained by transabdominal ultrasonography but a few were done transvaginally. Scans were obtained of both ovaries and any follicles ≥ 5 mm were measured and recorded. When several dimensions were measured, the average diameter of the follicle was used. It is unfortunate that only a few of our collaborating ultrasonologists were equipped with high resolution machines that permitted the reliable visualisation of cumulus within the follicle. Thus, in most cases we were not able to classify follicles with any confidence as dominant or atretic and could only use size as a guide.

RESULTS

The distributions of age, parity, height and weight in the two groups of mothers are compared in Table 1. The samples are similar in these respects except that the mothers of DZ twins are a little taller, as has been found by others [5].

The raw data from ultrasound scans are presented in the Figure. Scans where no follicle ≥ 5 mm was seen are left blank, as are scans missed for other reasons. Follicles are scored as 5-9 mm, 10-11 mm, 12-14 mm and ≥ 15 mm in average diameter. We have analysed the data using two size criteria for a dominant follicle – (a) at least 12 mm di-

MOTHERS OF DZ TWINS



(continued)

Figure. Results of ovarian ultrasound scans of 21 mothers of DZ twins and 18 controls including 13 mothers of MZ twins and 5 mothers of singletons. Each vertical bar represents a subject and each cross bar a cycle. Symbols to the left of the bar represent follicles seen in the left ovary and those to the right represent follicles in the right ovary. Where more than one measurement was taken of a follicle, its size class is determined by its average diameter.

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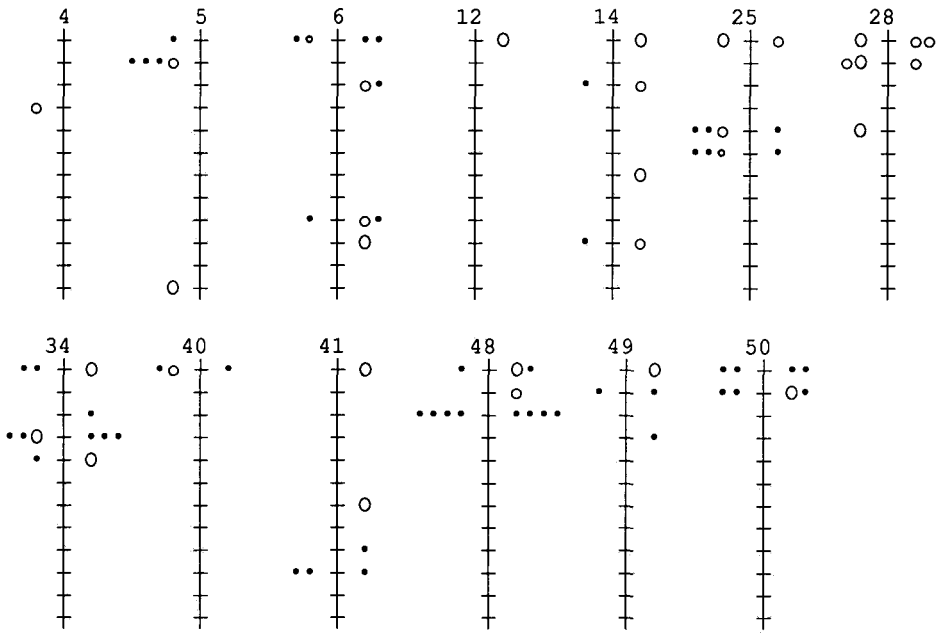
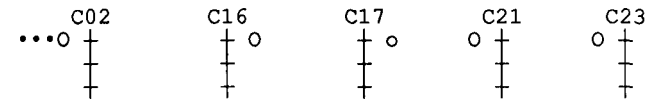
CONTROLS: MOTHERS OF MZ TWINS



CONTROLS: MOTHERS OF SINGLETONS



• = <10 mm ∘ = 10-11 mm, o = 12-15 mm, O = >15 mm.

Figure. *Continued*

Table 1 - Characteristics of mothers of DZ twins and control groups

	Mothers of DZ twins (N=21)		Controls (N=18)	
	Mean	SD	Mean	SD
Age	35.2	4.6	32.8	5.7
Number of pregnancies	3.2	1.3	2.8	1.7
Height (cm)	167.3*	5.6	162.8*	7.7
Weight (kg)	63.4	7.3	60.9*	10.8

* Mean heights differ. P=0.047.

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ameter, and (b) at least 15 mm diameter. Firstly we have classified each subject as a "single ovulator" or "double ovulator" according to whether we have scored any of her studied cycles as having at least two follicles fulfilling the size criterion. Secondly we have counted all eligible cycles (i.e. with at least one follicle of the required size) across all women and have compared the number of "single cycles" and "double cycles" in mothers of DZ twins and controls, although since several observations are made on the same person, these observations are not stricly independent. Since we have a strong prior hypothesis we are entitled to perform a one-tailed test of proportionality using Fisher's Exact Test. It can be seen (Table 2) that there is a significant excess of "double ovulators" among mothers of DZ twins compared with controls, and that in a significantly greater proportion of their cycles two follicles ripen instead of one. These results hold for both size criteria for dominant follicles.

We have also calculated the mean number of follicles per cycle for each woman, counting only those cycles and follicles which reach a given size criterion. For the 12 mm criterion, the unweighted average of these means for the mothers of DZ twins is 1.34 follicles per cycle with a standard deviation of 0.49, and for the controls 1.10 (SD 0.32). The average for DZ mothers is higher than for controls ($t_{35} = 1.80$, $P < 0.05$, 1 tail). Using the 15 mm criterion, the average value of follicles per cycle in DZ mothers is 1.20 (SD 0.39), while all eligible controls have a mean of one follicle per cycle. This difference too is significant ($t_{18} = 2.24$, $P < 0.05$).

However, there is a potential source of bias in our results. It can be seen that, on average, scans were obtained in 3.7 cycles for each mother of DZ twins while only 1.7 scans were performed for each control. Thus there were more opportunities for a mother of DZ twins than a control to be scored as a double ovulator. This bias is the more serious since we know that subjects who were found to be super-ovulatory at one scan were more cooperative and likely to return the next month for another. One rather extreme way to eliminate this bias is only to score a subject as a double or single ovulator on the basis of the very first scan for which she presented. This analysis has been done for both size criteria (Table 3) and in neither case is there now a significant difference in the proportion of double ovulators between mothers of DZ twins and controls, although the direction of the effect is consistent with our hypothesis. However, this correction is probably excessively conservative and, considered as a whole, our data point very strongly to much higher average follicular activity in women who have previously had spontaneous DZ twins than control mothers.

Table 2 - Results of ovarian ultrasound scans for mothers of DZ twins compared with control mothers of MZ twins and singletons (MZ+S). Cycles in which two follicles are seen fulfilling the size criterion (≥12 mm or ≥15 mm) are classed as "double cycles" and women with at least one such cycle are classed as "double ovulators". One-tail probabilities as calculated by Fisher's exact test

Follicles ≥12 mm

	Mothers of MZ+S	DZ	
Double ovulators	2	13	15
Single ovulators	16	8	24
	18	21	39
	P=0.001		

	Mothers of MZ+S	DZ	
Double cycles	3	24	27
Single cycles	28	53	81
	31	77	108
	P=0.015		

Follicles ≥15 mm

	Mothers of MZ+S	DZ	
Double ovulators	0	7	7
Single ovulators	15	12	27
	15	19	34
	P=0.009		

	Mothers of MZ+S	DZ	
Double cycles	0	15	15
Single cycles	21	43	64
	21	58	79
	P=0.005		

Table 3 - Classification of women as double or single ovulators using the result of only the first valid scan i.e. the first scan in which at least one follicle fulfilled the size criterion (≥12 mm or ≥15 mm). One-tail probabilities by Fisher's exact test

First scan ≥12 mm

	Mothers of MZ+S	DZ	
Double ovulators	2	5	7
Single ovulators	16	16	32
	18	21	39
	P=0.12		

First scan ≥15 mm

	Mothers of MZ+S	DZ	
Double cycles	0	3	3
Single cycles	15	16	31
	15	19	34
	P=0.16		

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DISCUSSION

Even though some women have two or more sets of DZ twins, one would be surprised if multiple ovulation occurred in all their cycles. Little is known about the frequency or pattern of multiple ovulation in these women, or in women who never have twins. We have used ultrasonography to examine ovaries in late-follicular phase in a sample of mothers of spontaneous DZ twins and controls. Controls were mainly mothers of MZ twins. All women were cycling regularly and were not using oral contraceptives. We observed multiple large follicles at cycle day 12 significantly more frequently in mothers of DZ twins (13/21 mothers, 24/77 cycles) than controls (2/18 mothers, 3/31 cycles). This is equivalent to a sensitivity of 62% and a specificity of 89%. For some mothers we have obtained scans in up to six successive cycles and multiple large follicles have been observed in several of them.

The frequency of multiple large follicles in controls is in line with the few previous reports of double ovulation in unselected, naturally cycling women; Queenan et al [22] reported 2/18 women with double ovulation, O'Herlihy et al [17] observed 3 instance double ovulation in 53 cycles of 33 women, and Potashnik et al [21] saw double ovulation in 7/90 cycles of 16 women. Summing our results with those from these three studies gives an estimate of 8% (15/192) for the frequency of double ovulation in cycles of normal women versus 31% (24/77) in women who have already had natural DZ twins – a fourfold difference in risk. These risks are crude averages and ignore heterogeneity of the individual risk between women within groups which is apparent from the Figure. Nevertheless, our results support the notion that dizygotic pregnancy is not a chance event but happens predominantly in women who are predisposed to multiple ovulation, presumably by hormonal profiles which are partly genetically determined.

Double ovulation can occur when two eggs are released from one ovary or one from each. The two cases of double ovulation reported by Queenan et al [22] were both bilateral, while the seven instances reported by Potashnik et al [21] were all in the same ovary (unilateral). Of the 27 instances of multiple large ($\geq$ 12 mm) follicles we have observed, ten were in the same ovary, nine in opposite ovaries and in eight instances we saw three or more follicles distributed between both ovaries (ambilateral). In one woman (no. 10) we saw all three patterns in different cycles, in two further women (no. 16, no. 20) we observed both uni-and bilateral patterns, and in another (no. 29) both bi-and ambilateral patterns were seen.

Of the 97 follicles observed by Potashnik et al [21], significantly more (64%) were seen in the right ovary than the left and they speculate that differences in vasculature of the ovaries may bias ovulation to occur more frequently from the right ovary. In our control sample, 22/36 (61%) of follicles ($\geq$ 12mm) were seen in the right ovary although the difference was not significant, but in the mothers of DZ twins, 51% (57/112) of follicles were seen in each ovary. If there is a bias toward right-sided ovulation in the general population, it seems not to operate in women predisposed to multiple ovulation. Of the ten instances of unilateral multiple large follicles in eight women, eight occurred in the left ovary in six women and two on the right in two women.

Related to the question of laterality of multiple ovulation in predisposed mothers and bias in laterality of ovulation in the general population of women, is the question

of sequence. Over successive cycles does ovulation tend to alternate between ovaries (contralateral) or keep occurring in the same ovary (ipsilateral)? Marinho et al [11] presented ultrasound evidence that ovulation occurs from alternate ovaries in 80% of healthy subjects with regular menses and Gougeon and Lefevre [7] obtained a similar estimate (88%) by evaluating degenerating corpora lutea from 113 cycles in the removed ovaries of 25 women. Potashnik et al [21] collected ultrasound data over a longer series of cycles and found alternation in only 24%. They suggest that if the follicular phase is short (≤ 14 d) "the ovary still seems to be 'suppressed' by the activity of the previous cycle, and therefore ovulation tends to occur on the other side. When the follicular phase is prolonged, on the other hand, the ovary may have sufficient time to free itself from this inhibitory effect, allowing ovulation to occur on the same side". Their results echo those of an earlier study on rhesus monkeys [24] in which the authors suggest that "the corpus luteum of the previous cycle provides sufficient residual local activity to designate which ovary will provide the follicle for ovulation in the subsequent cycle". Our data are certainly not ideal to cast further light on this question but inspection of the Figure suggests no regular features of lateral control of ovulation in women predisposed to twinning. If there are paracrine factors regulating the side of ovulation and the number of dominant follicles in each ovary [16,9,23], they appear to be overridden by more powerful, presumably endocrine factors in mothers of twins. However, a very recent study [3] of 286 pairs of ovulation observed in 92 women fails to find any systematic evidence for ipsilateral over contralateral, and the authors suggest that the side of ovulation in a given cycle is random.

There are several limitations of our data: (a) the data for number of double ovulatory cycles may be biassed because those women in whom we saw multiple follicles once tended to keep coming back for more scans while those in whom we did not tended to drop out of the study earlier; (b) to assemble the data presented here, over 140 scans were taken by 27 ultrasonologists from 13 clinics in 6 cities. There is thus a danger that the scans were of uneven quality and the data correspondingly unreliable. It is also possible that operators tended to look harder for multiple follicles in mothers of DZ twins than controls. It would have been preferable if our study could be have been done blind in this respect, but from a practical point of view this was difficult; (c) it is hard to define what may be classified as a "dominant follicle" since even large follicles may become atretic [1]. We have presented results using both 12 mm and 15 mm average diameter as the criterion for a dominant follicle since these seem to encompass most views of what indicates dominance [22,17,1,6] but different criteria will alter the numbers given above. We aimed to scan on day 12 of a regular 28 day cycle, but this varied if it fell on a weekend, if the cycle length was usually longer or shorter than this, and for numerous other practical reasons. All these factors make definition of a "dominant follicle" harder and our data correspondingly less reliable. Of particular concern was our inability to screen out atretic follicles, since Gougeon and Lefevre [6] claim that atretic follicles > 9 mm diameter may often be seen in follicular phase, although Bomsel-Helmreich [1] found that no follicle that did not subsequently ovulate was seen to be larger than 14-16 mm. Ideally, all follicles should have been followed through to ovulation, but this was impractical in this study. Failing that, scans should be late enough in follicular phase and of sufficient quality to visualise the cumulus which is present in follicles destined to ovulate, but once again this was not always possible. Even then, Bomsel-Helmreich

[1] claims that atretic follicles are visually very similar to preovulatory follicles and that a single sonographic image cannot distinguish between them.

We are well aware that an ideal study would have had a the sample of mothers of DZ twins and matched controls (mothers of singletons only) scanned by a single sonographer in a blinded fashion, all over the same number of cycles. But our subjects were all volunteers without any clinical indications and we were not able to offer any financial or other inducements for cooperation. There was not a large enough number in any one centre, and even if there had been, no single sonographer was available who could perform the number of scans required and fulfil his clinical obligations. We hope that our interesting results will stimulate someone with better resources to do the study properly.

Despite all the potential shortcomings of our study, we are confident that our data present a strong case for much greater follicular activity than average in mothers of DZ twins, such that multiple ovulation is a much more frequent event than in women of average fertility. Elsewhere we present evidence that mothers of DZ twins have very much higher early-follicular levels of LH and FSH than controls and higher mid-follicular inhibin and estradiol levels [13,14]. We argue that this points to pituitary or hypothalamic control of the (probably hereditary) tendency to multiple ovulation in humans. If this is the main underlying mechanism of human multiple ovulation, it would be consistent with the apparent lack of evidence for paracrine control from our ultrasound data.

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REFERENCES

1. Bomsel-Helmreich O (1985): Ultrasound and the preovulatory human follicle. In J.R. Clarke (ed): Oxford Reviews of Reproductive Biology. London: Clarendon Press, pp. 1-72.
2. Bulmer MG (1970): The Biology of Twinning in Man. Oxford: Oxford University Press.
3. Check JH, Dietterich C, Houck MA (1991): Ipsilateral versus contralateral ovary selection of dominant follicle in succeeding cycle. Obstet Gynecol 77: 247

4. Chen P, Hayward NK, Kidson C, Ellem KAO (1990): Conditions for generating well- resolved human DNA fingerprints using M13 phage DNA. Nucleic Acids Res 18:1065.
5. Corney G, Seedburgh D, Thompson B, Campbell DM, MacGillivray I, Timlin D (1981): Multiple and singleton pregnancy: differences between mothers as well as offspring. In L Gedda, P Parisi, W Nance: Twin Research 3: Twin Biology and Multiple Pregnancy. New York: AR Liss Inc, pp 107-114.
6. Gougeon A, Lefevre B (1983): Evolution of the diameters of the largest healthy and atretic follicles during the human menstrual cycle. J Reprod Fert 69:497-502.
7. Gougeon A, Lefevre B (1984): Histological evidence of alternating ovulation in women. J Reprod Fert 70:7-13.
8. Haukioja E, Lemmetyinen R, Pikkola M (1989): Why are twins so rare in *Homo sapiens*? Amer Nat 133:572-577.
9. Hodgen GD (1986): Ovarian function for multiple follicle maturation. Clin Obstet Gynaecol 29:127-140.
10. Khoury MJ, Erickson JD (1983): Maternal factors in dizygotic twinning: Evidence from interracial crosses. Ann Hum Biol 10:409-416.
11. Marinho AO, Sallam HN, Goessens L, Collins WP, Campbell S (1982): Ovulation side and occurrence of mittelschmerz in spontaneous and induced ovarian cycles. Br Med J 284:632.
12. Martin NG, Martin PG (1975): The inheritance of scholastic abilities in a sample of twins I. Ascertainment of the sample and diagnosis of zygosity. Ann Hum Genet 39:213-218.
13. Martin NG, Robertson DM, Chenevix-Trench G, de Kretser DM, Osborne J, Burger HG (1991): Elevation of follicular phase inhibin and luteinizing hormone levels in mothers of dizygotic twins suggests non-ovarian control of human multiple ovulation. Fertil Steril 56:469-474.
14. Martin NG, Olsen ME, Thiele H, El Beaini JL, Handelsman D, Bhatnagar AS (1984): Pituitary-ovarian function in mothers who have had two sets of DZ twins. Fertil Steril 41:878-880.
15. Mather K (1973): The Genetical Structure of Populations. London: Chapman and Hall.
16. Nilsson L, Wikland M, Hamberger L (1982): Recruitment of an ovulatory follicle in the human following follicle-ectomy and luteectomy. Fertil Steril 37:30-34.
17. O'Herlihy C, de Crespigny LJ, Robertson HP (1980): Monitoring ovarian follicular development with real-time ultrasound. Br J Obstet Gynaecol 87:613-618.
18. Parisi P, Gatti M, Prinzi G, Caperna G (1983): Familial incidence of twinning. Nature 304:626-8.
19. Philippe P (1985): Genetic epidemiology of twinning: A population-based study. Am J Med Genet 20:97-105.
20. Philippe P, Roy R (1989): Conceptive delays of twin-prone mothers: A demographic epidemiologic approach. Hum Biol 61:599-614.
21. Potashnik G, Insler V, Meizner I, Sternberg M (1987): Frequency, sequence and side of ovulation in women menstruating normally. Br Med J 294:219.
22. Queenan JT, O'Brien GD, Bains LM, Simpson J, Collins WP, Campbell S (1980): Ultrasound scanning of ovaries to detect ovulation in women. Fertil Steril 34:99-105.
23. Tonetta SA, di Zerega GS (1986): Paracrine regulation of follicular maturation in primates. Clin Endocrinol Metab 15:135-156.
24. Wallach EE, Virutamasen P, Wright KH (1973): Menstrual cycle characteristics and side of ovulation in the rhesus monkey. Fertil Steril 24:715-721.

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