

# The Control of Ovulation in Mothers of Dizygotic Twins\*

CHRISTOPHER P. GILFILLAN, DAVID M. ROBERTSON, HENRY G. BURGER,  
MARIA A. LEONI, VICTOR A. HURLEY, AND NICHOLAS G. MARTIN

Prince Henry's Institute of Medical Research and Monash Ultrasound for Women (M.A.L., V.A.H.),  
Clayton, Victoria 3168; and Queensland Institute of Medical Research (N.G.M.), Herston,  
Queensland 4006, Australia

## ABSTRACT

Dizygotic twinning is familial, suggesting that there may be an inherited abnormality of the control of ovulation that predisposes to double ovulation and, therefore, dizygotic twins. The present study examines 17 mothers of dizygotic twins (MODZT) and 8 control mothers of singletons by daily blood sampling throughout an entire menstrual cycle. Blood samples were assayed for LH, FSH, estradiol, progesterone, and inhibin. The process of follicular development was followed by transvaginal ultrasound. The pituitary LH response to iv GnRH was also assessed. Three of the 16 MODZT double ovulated during the study compared to none of the 8 control mothers ( $P < 0.05$ ). The number of small follicles ( $< 6$  mm) declined significantly in control

women at midcycle, but not in MODZT. There was no significant difference in serum FSH, LH, estradiol, or inhibin levels between the 2 groups at any stage of the menstrual cycle. During the follicular phase, serum progesterone levels were significantly higher in MODZT. The response to GnRH stimulation was not different between MODZT and controls. In conclusion, this study demonstrates an increased tendency to double ovulate in MODZT that may be due to a reduced rate of atresia in advanced follicles. Furthermore, the elevated progesterone levels in MODZT during the follicular phase suggest altered intrafollicular steroidogenesis that is independent of gonadotropins. (*J Clin Endocrinol Metab* 81: 1557–1562, 1996)

THE TENDENCY to conceive spontaneous dizygotic twins is familial and varies across racial groups, suggesting a genetic predisposition (1–3), although the mode of inheritance is far from clear (1, 4). There are also important environmental factors, such as age and parity of the mother, and nutritional status. Mothers of dizygotic twins (MODZT) have a higher incidence of spontaneous multiple ovulations than control women (5), and as gonadotropin therapy in women leads to multiple ovulation, we hypothesized that elevated gonadotropin levels might be the proximate cause of twinning in genetically predisposed women. Earlier studies by Nylander (6) had shown nonsignificant elevations of FSH at midcycle in African MODZT compared with control mothers of singletons, whereas further studies failed to show significant FSH elevations in MODZT (7). More recent studies concentrated on the early follicular phase and demonstrated elevated FSH, LH, estradiol, and inhibin levels in MODZT (8, 9), suggesting that pituitary overproduction of gonadotropins at the time of follicular recruitment was the primary event, rather than deficiency of known ovarian regulators of gonadotropin secretion (inhibin and ovarian steroids). These studies did not establish whether women double ovulated during the study and, therefore, could not exclude the possibility that hormonal differences were secondary to double ovulation *per se*. Furthermore, if pituitary gonadotropin secretion is enhanced in MODZT, this could be secondary to enhanced pituitary sensitivity to GnRH, but no

study of the pituitary response to GnRH in MODZT has been reported. It is also not known whether multiple ovulation in MODZT results from recruitment of a larger cohort of follicles early in the cycle or whether there is an abnormality of selection of the dominant follicle(s) later in the follicular phase. No detailed ultrasound study of follicular development in MODZT throughout an entire cycle has been reported.

Three hypotheses were tested: 1) that differences in peripheral hormone levels between MODZT and controls will be apparent during a monoovulatory cycle and may, therefore, reflect the primary dysregulation that leads to double ovulation; 2) that increased FSH drive to the process of follicular development is a consequence of increased pituitary responsiveness to GnRH; and 3) that the increased tendency to double ovulation in MODZT will be a consequence of a larger number of follicles recruited to the developing pool in the early follicular phase.

To test these hypotheses, we studied a group of MODZT and an age-matched control group of mothers of singletons. The study consisted of three parts: 1) examination of the process of follicular development by transvaginal ultrasound, 2) detailed study of the peripheral concentrations of the reproductive hormones during an entire menstrual cycle by daily blood sampling, and 3) determination of the LH response to GnRH administration.

## Subjects and Methods

### Subjects

Subjects were recruited through the mothers of twins clubs of Melbourne (affiliated with the Australian Multiple Births Association) by advertising in newsletters and by public seminars. Control women were generally friends of the subjects. Seventeen MODZT comprise the subject group. These were mothers of one set of dizygotic twins who had never been exposed to gonadotropins or clomiphene citrate, were hav-

Received June 6, 1995. Revision received November 3, 1995. Accepted December 5, 1995.

Address all correspondence and requests for reprints to: Dr. C. P. Gilfillan, Prince Henry's Institute of Medical Research, P.O. Box 5152, Clayton, Victoria 3168, Australia.

\* This work was supported by the National Health and Medical Research Council of Australia.

ing regular menstrual cycles, were less than 40 yr old, and had not been taking oral contraceptives for 12 months. Dizygosity of the twins was established by the presence of clear differences in sex, eye color, or hair color and, where doubt existed, by DNA fingerprinting (10). Sixteen MODZT had a positive family history of twinning, and five had dizygotic twins in either their own or their parent's sibships. Eight mothers of singletons comprised the control group. These women also had regular menstrual cycles and were less than 40 yr of age. Two of these women also had a positive family history of twinning, although not in first degree relatives. All study participants gave their informed consent. The baseline characteristics of the two groups are shown in Table 1. There were no significant differences between the two groups with respect to age, parity, cycle characteristics, or anthropometric variables. There was a tendency for the reported age at menarche to be earlier in MODZT, but this also failed to reach significance. The average age at conception of twin pregnancies was 29.5 yr. Family histories of the study participants were scored, with a twin pregnancy in a first degree relative (mothers and sisters) scoring 3 points, in a second degree relative (grandmothers, aunts, or cousins) 2 points, and more distant relatives 1 point. The mean score in MODZT ( $3.12 \pm 2.2$ ) was significantly higher than that in controls ( $0.63 \pm 1.41$ ;  $P < 0.05$ ).

### Study protocol

Entry into the study was coordinated by measuring urinary LH at home with a rapid LH home test kit (ClearPlan, Unipath, Bedford, UK). After detection of the LH peak, blood sampling began at LH peak plus 8 days. Daily blood samples (15 mL) were collected at home by a visiting nurse, generally in the morning. Sampling continued from LH peak +8 of cycle 1 through the onset of menstruation throughout an entire cycle until 2 days after the start of the second menstrual bleed. This was a mean total of 37 samples/subject. Samples were allowed to clot overnight, then were spun, and serum was decanted and stored in three aliquots at  $-20^\circ\text{C}$  until assayed.

Transvaginal ultrasound was performed as nearly as practical to days 4, 8, 12, 14, and 21 of cycle 2, using an Acuson XP-10 ultrasound system (Acuson, San Francisco, CA) with a 5-megahertz vaginal transducer. One operator (M.A.L.) performed 90% of the observations using a standardized protocol and was blinded to the reproductive history of the study participants.

On day 2 or 3 of cycle 3, a GnRH stimulation study was performed. Blood samples (10 mL) were drawn before and 15, 30, 45, 60, 75, and 90 min after the administration of iv GnRH (100  $\mu\text{g}$ ; Relefact LH-RH, Hoechst, Frankfurt-Main, Germany). Blood samples were allowed to clot overnight, then were spun, and serum was decanted and stored at  $-20^\circ\text{C}$  until assayed. These samples were assayed for LH only.

The study protocol was approved by the Monash Medical Center human ethics committee in accordance with the guidelines of the Declaration of Helsinki.

### Assays

All serum samples were assayed in duplicate and in random order. Serum estradiol and progesterone were measured by solid phase RIA (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). The

estradiol range was 70–13,200 pmol/L, with an interassay coefficient of variation between 6.6–15.9% and an intraassay coefficient of variation between 4.6–12.3%. The progesterone range was 0.3–127 nmol/L, with an interassay coefficient of variation between 8.9–11.6% and an intraassay coefficient of variation between 5.2–6.7%. LH was measured by Microparticle Enzyme Immunoassay (LH-IMX, Abbott Laboratories, North Chicago, IL). The sensitivity of this assay was 0.5 IU/L, with an interassay coefficient of variation between 5.0–6.7% and an intraassay coefficient of variation between 3.1–5.5%. FSH was measured by two-site immunoenzymometric assay (AIA-Pak FSH, Tosoh, Tokyo, Japan). The sensitivity of this assay was 0.3 IU/L, with an interassay coefficient of variation between 5.0–5.9% and an intraassay coefficient of variation between 3.1–4.0%. Inhibin was measured by RIA using the Monash 1989 assay (11). The sensitivity of this assay was 37 IU/L, with an interassay coefficient of variation between 10–22%.

### Statistics

For the ultrasound study, the numbers of follicles observed per observation at each time interval was compared between groups by *t* test. The hormonal data were analyzed by ANOVA with repeated measures. The GnRH stimulation data were analyzed by ANOVA with repeated measures and area under the curve analysis.

## Results

### Transvaginal ultrasound

The distribution of follicles was determined by size (3–6, 7–10, 11–12, 13–15, and  $>15$  mm, and corpora lutea) in three phases of the menstrual cycle, designated cycle day  $<10$  (early to midfollicular phase), cycle days 11–16 (midcycle), and cycle day  $>16$  (luteal phase), based on the relationship to the day of the LH surge (cycle day 14; Fig. 1). The average number of follicles per observation in both ovaries is presented. In MODZT, the number of 3- to 6-mm follicles remained constant throughout the menstrual cycle, whereas in control women, there was a decline in the number of 3- to 6-mm follicles in the midcycle ( $P < 0.05$ ). Only a small number of follicles progressed beyond this size, and there were no significant differences between the two groups in the number of follicles observed at each size interval. Although the number of 7- to 10-mm follicles was lower in MODZT during the luteal phase (cycle day  $>10$ ), this was not significant ( $P = 0.056$ ).

All women developed at least 1 dominant follicle ( $>15$  mm), and in all but 3 women a corpus luteum was detected, although it would appear from the hormone data that all women ovulated. Three women (designated DO1, DO2, and DO3) in the subject group double ovulated, as evidenced by the presence of 2 corpora lutea, for 1 subject (DO1) in contralateral ovaries and for 2 subjects (DO2 and DO3) in the same ovary. The numbers of smaller follicles in these women were not different from those in the remainder of the MODZT group, and exclusion of these women from the analysis did not alter the conclusion. The double ovulation rate in MODZT was not significantly greater than that in controls (by  $\chi^2$  test,  $P = 0.2$ ). No woman destined to ovulate singly developed more than 1 follicle that was more than 15 mm. Ovarian volumes were greatest on the side destined to ovulate, but were not different between the groups. There were 16 ovulations on the left side and 10 on the right.

### Hormonal studies

The hormonal data from each subject were organized about two reference points: the first day of menstrual bleed-

**TABLE 1.** Baseline characteristics (mean  $\pm$  SD) of MODZT and controls

	Controls	MODZT	P value
No. of subjects	8	17	
Age (yr)	$33.8 \pm 2.9$	$33.7 \pm 3.3$	NS
Ht (m)	$1.65 \pm 0.07$	$1.65 \pm 0.09$	NS
Wt (kg)	$73.2 \pm 19.5$	$71.9 \pm 15.9$	NS
Body mass index ( $\text{kg}/\text{m}^2$ )	$26.6 \pm 5.2$	$26.6 \pm 6.0$	NS
Cycle (days)	$27.1 \pm 3.4$	$27.8 \pm 2.3$	NS
Age at menarche (yr)	$13.6 \pm 2.1$	$12.7 \pm 1.0$	NS
Parity	$2.1 \pm 0.6$	$2.5 \pm 0.9$	NS
Age at conception (yr)		$29.5 \pm 2.5$	
Family history score <sup>a</sup>	$0.6 \pm 1.4$	$3.1 \pm 2.2$	$<0.05$

<sup>a</sup> Family history score for twin conceptions: first degree relative = 3; second degree relative = 2; and more distant history = 1.

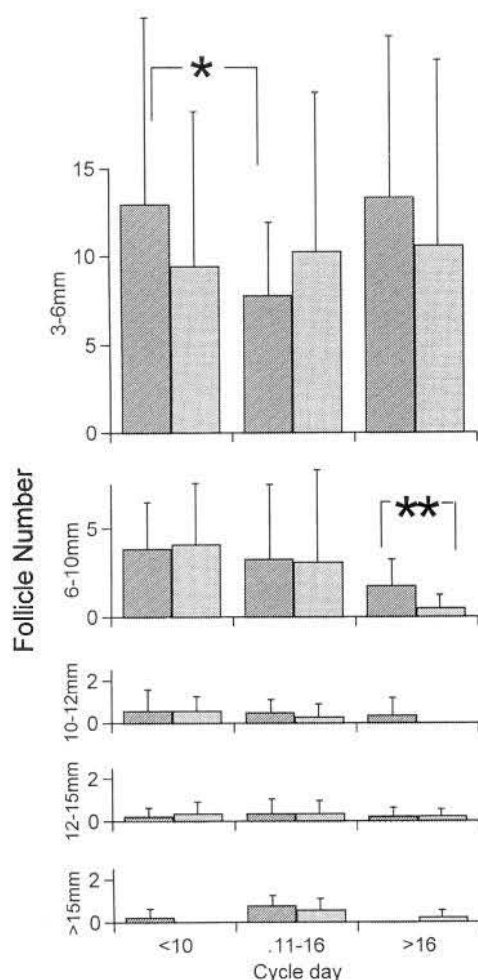


FIG. 1. The distribution of follicles, grouped by size, during three phases of the menstrual cycle for control women (dark bars) and MODZT (light bars). The mean number of follicles in each size grouping per observation for each individual during each phase of the menstrual cycle has been combined in a group mean  $\pm$  SD for each study group. \*,  $P < 0.05$ ; \*\*,  $P = 0.056$ .

ing and the midcycle LH surge. This enabled individual data to be combined as the group mean  $\pm$  SD for each cycle day. Individual data series were also combined in an ANOVA with repeated measures when looking for a significant effect of study group. Data from the three women who double ovulated were excluded from the analysis and are examined individually below.

#### Single ovulations

Serum FSH was elevated during the late luteal and early follicular phases, with a decline in the late follicular phase and a second rise at the time of the LH peak (Fig. 2A). There was no significant difference between the two study groups at any stage of the menstrual cycle. Serum estradiol increased throughout the follicular phase and declined after the LH surge, with a secondary rise during the luteal phase. Again, there was no significant difference between the two study groups at any stage of the menstrual cycle (Fig. 2B). Serum inhibin rose briefly at the time of the LH surge and again during the luteal phase, only to fall at the end of the luteal

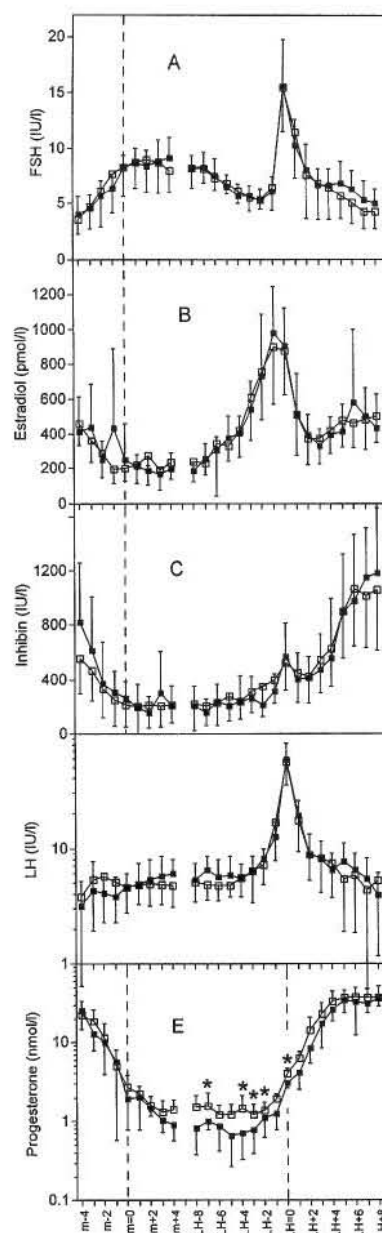


FIG. 2. Hormonal data (mean  $\pm$  SD) for MODZT ( $\square$ ) and controls ( $\blacksquare$ ) arranged around the first day of menstruation (M = 0) and the day of the LH surge (LH = 0). A, FSH (international units per L); B, estradiol (picomoles per L); C, inhibin (international units per L); D, LH (international units per L); E, progesterone (nanomoles per L). \*,  $P < 0.05$  for a significant effect of study group.

phase and remain at relatively low levels during the follicular phase. Serum inhibin did not differ significantly between the two groups at any stage of the menstrual cycle (Fig. 2C). Serum LH showed a trend to be lower in MODZT during the follicular phase, but this was not significant (Fig. 2D).

Serum progesterone was significantly higher in MODZT during the follicular phase ( $P = 0.021$ ), but not the luteal phase, and many comparisons on individual days reached significance (Fig. 2E). The mean follicular phase progesterone level in MODZT was  $1.59 \pm 0.71$  nmol/L compared with  $1.11 \pm 0.71$  nmol/L in controls. The ratio of LH to progesterone

terone was significantly lower in MODZT during the follicular phase ( $P = 0.006$ ), with comparisons on individual days again reaching significance (Fig. 3).

#### Double ovulations

Of the three MODZT who double ovulated (DO1–3), DO1 had markedly elevated FSH levels in the postmenopausal range, a pattern distinctly different from those in DO2 and DO3, who had low FSH levels throughout the menstrual cycle (Fig. 4A). DO1 also had elevated LH levels and relatively low inhibin and progesterone levels (Fig. 4, B–D), whereas DO2 and DO3 had low LH levels not only in the luteal phase but also in the early follicular phase and relatively high inhibin and progesterone levels (Fig. 4, B–D). The serum estradiol level was not distinguishable from that in the control group in any of the women who double ovulated (data not shown).

#### GnRH stimulation test

In response to iv GnRH (100  $\mu$ g), serum LH increased by 300% and peaked at 30 min in both groups (Fig. 5). There was no significant difference between the responses in the two groups, whether determined by ANOVA with repeated measures or by area under the curve analysis.

#### Discussion

We studied 17 MODZT and compared them to 8 mothers of singletons as controls in an attempt to determine the mechanism of multiple ovulation in MODZT. We hypothesized that an inherited tendency to conceive DZT would be reflected by dysregulation in monoovulatory cycles in MODZT. Evidence for dysregulation could be in the form of an altered hormonal environment or alterations in populations of follicles within the ovary. Hormonal differences between MODZT who double ovulate and controls may be either primary or secondary to the development of multiple

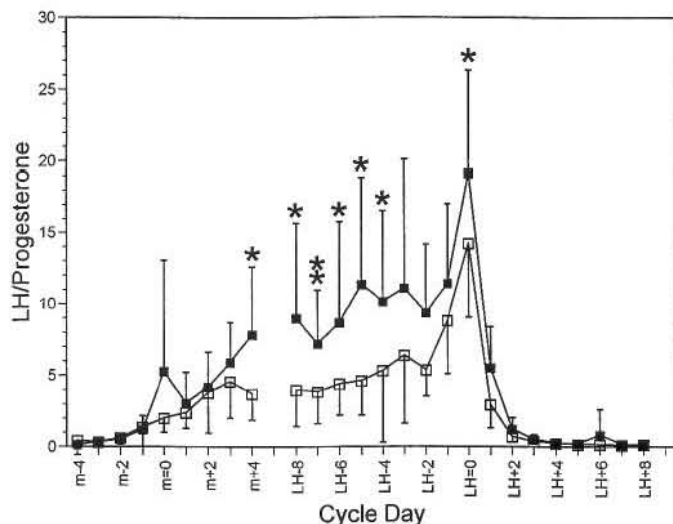


FIG. 3. Serum LH/progesterone ratio (mean  $\pm$  SD) for MODZT (■) and controls (□) throughout the study cycle. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (for difference between groups, determined by ANOVA with repeated measures).

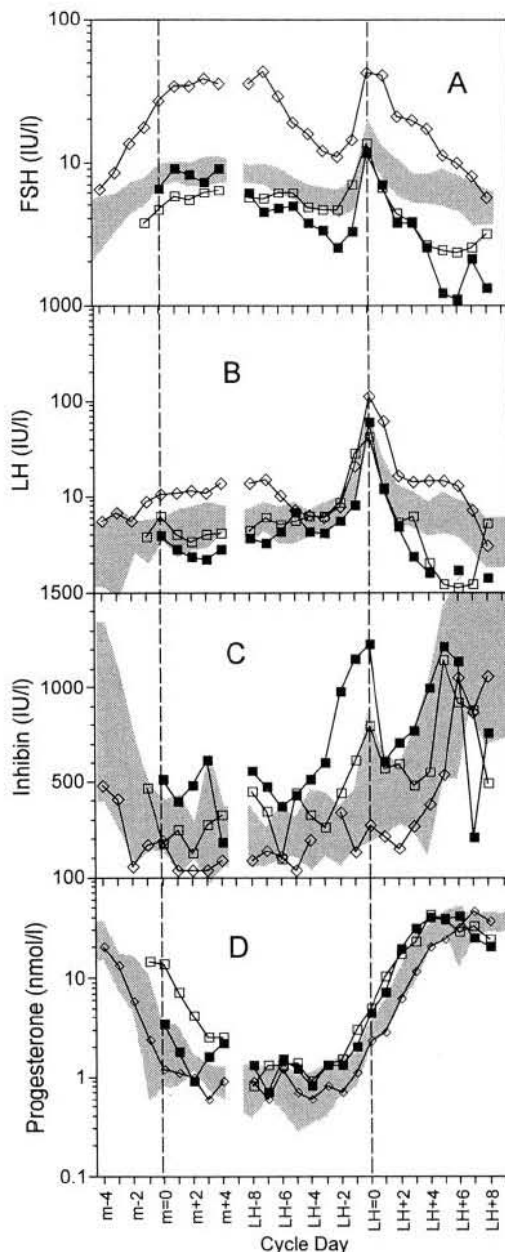


FIG. 4. Hormonal profiles of the three women who double ovulated [DO1 (◇), DO2 (□), DO3 (■)] compared to control data  $\pm$  1 SD (shaded area). A, FSH (international units per L); B, LH (international units per L); C, inhibin (international units per L); D, progesterone (nanomoles per L).

large follicles, but may again reflect altered follicular regulation.

In the human, follicular development begins with an initial FSH-independent phase followed by recruitment of a cohort of follicles by rising FSH levels that develop during the late luteal phase of the preceding cycle. Progressively, follicles are diverted to atresia by a mechanism that is poorly understood (12). The emergence of one dominant follicle is usually evident by the early follicular phase, identified by both size and differential steroid secretion, with elevated progesterone levels in the ovarian vein draining the ovary

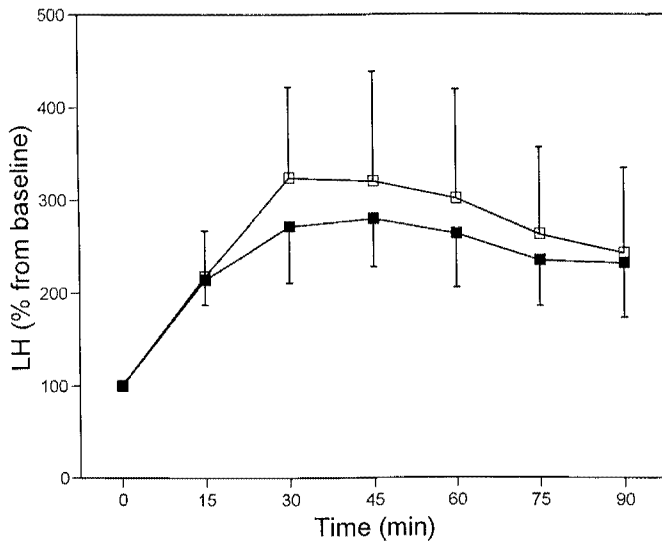


FIG. 5. Serum LH after iv administration of GnRH (100  $\mu$ g; Relefact LH-RH, Hoechst) in controls (■) and MODZT (□). Values are expressed as a percentage of the baseline (time zero)  $\pm$  1 SD.

that will ultimately ovulate evident as early as day 1 of the cycle (13). The dominant follicle grows throughout the follicular phase and progressively acquires differentiated functions, such as progesterone production. Ovulation then follows the LH surge at midcycle (14). Nondominant follicles are rarely greater than 10 mm, but earlier work showed MODZT to have more than one follicle of more than 15 mm in up to 30% of cycles compared with mothers of singletons, who rarely do (5). In our study MODZT were defined as double ovulators only if two corpora lutea could be identified on day 21. This more stringent criterion provided a double ovulation rate of about 18% (3 of 17).

Double ovulation could result from either recruitment of a larger cohort of small follicles, or alternatively, the recruited cohort could be of normal size, but the process of atresia and selection could be impaired. Our data from the ultrasound study support the latter contention. Despite the fact that 3 of the 17 MODZT double ovulated in the study cycle, there was no excess of small and medium-sized follicles seen by transvaginal ultrasound in the MODZT during the early follicular phase, suggesting that the recruited cohort was similar in number in the 2 groups. At midcycle, the number of small follicles declined significantly in controls, but this did not occur in MODZT, suggesting that follicles in MODZT may be more resistant to the process of selection and atresia than follicles in control women. This picture is in contrast to that observed during human menopausal gonadotropin treatment of women, where gonadotropin excess induces large numbers of small and medium-sized follicles to enter the developing pool, many of which reach maturity (15).

In MODZT who did not double ovulate during the study cycle, FSH and LH levels were indistinguishable from those in controls. There was a trend for lower LH levels during the follicular phase and lower FSH levels during the late luteal phase in MODZT, but neither reached significance. This is in conflict with earlier work that demonstrated elevated gonadotropin levels in the early follicular phase (8, 9). In these

studies smaller numbers of subjects were examined over a shorter period, and no attempt was made to exclude women who double ovulated during the study cycle. The midcycle elevations of FSH described by Nylander in Nigerian MODZT failed to reach statistical significance (6, 7). Inhibin and estradiol levels were not different between monoovulating MODZT and controls. The finding of elevated inhibin and estradiol levels in MODZT during the early follicular phase in an earlier study (9) may have been due to the inclusion of women who double ovulated.

As the pituitary LH response to supraphysiological GnRH was not different between groups, we conclude that the maximal LH secretory response is similar in MODZT and controls. The present study does not exclude the possibility that MODZT have altered sensitivity to physiological GnRH pulses.

In monoovulating MODZT, follicular phase progesterone levels were elevated by 50% compared to control values. The LH to progesterone ratio exaggerated the difference between groups, reflecting the reciprocal relationship between these two hormones in this setting. Progesterone production, initially gonadotropin independent (16) and later in response to LH (17), is a differentiated function of granulosa cells and is lost in atretic follicles (18). Progesterone levels are higher in ovarian venous blood from the ovary destined to ovulate even in the early follicular phase. These findings suggest that progesterone production may be a marker of dominance and may be elevated in women with more follicles displaying a dominant phenotype. In addition, progesterone receptor messenger ribonucleic acid is present in the ovary (19), suggesting that intrafollicular progesterone may have a paracrine role within the ovary. Progesterone has been postulated to have a role in the process of ovulation (20, 21), oocyte maturation, and the development of the corpus luteum (22). The activin IIb receptor gene has recently been reported to have a progesterone receptor recognition sequence in its promoter region (23), suggesting that progesterone may be able to modulate activin action within the follicle (24). We would conclude, therefore, that MODZT have follicles that produce more progesterone, probably by a gonadotropin-independent mechanism. This may contribute to a relative resistance to atresia, possibly by promoting activin action within the follicle. Alternatively, increased progesterone production may be an epiphenomenon, reflecting an increased number of follicles exhibiting a dominant phenotype.

We also had the opportunity to compare late luteal phase hormone levels in two consecutive cycles in MODZT and controls. Increased variability in hormone levels from cycle to cycle might give an index of dysregulation in MODZT; however, there was no significant differences in the variation in hormone levels on the same day in consecutive cycles between the two groups.

In two of the MODZT who did double ovulate (DO2 and DO3), gonadotropin levels were reduced throughout the entire cycle, suggesting that the process of follicular development was being driven independently of gonadotropins. These women had higher inhibin and estradiol levels than controls, consistent with the development of two dominant follicles and two corpora lutea. These findings again argue

against a primary role for gonadotropin elevations in the etiology of multiple ovulation in all MODZT.

In the third woman who double ovulated (DO1), gonadotropin levels were elevated into the postmenopausal range, although at the time she was only 31 yr of age and has continued to have regular cycles over 3 yr of follow-up. This suggests that there may be a second mechanism for double ovulation in MODZT, which is not observed in the majority of MODZT in this study, where elevated gonadotropins promote follicular development. It is known that gonadotropin levels increase with age and that significant elevations can occur in particular cycles and may precede the menopause by up to 10 yr (25). It may be this mechanism that accounts in part for the increasing incidence of dizygotic twins with advancing maternal age at conception. However, the increase in the dizygotic twinning rate with age precedes the rise in FSH by at least 10 yr, suggesting that other, perhaps intragonadal, mechanisms are at work (1). Despite the fact that two lead follicles were developing, this subject had distinctly low inhibin levels throughout the cycle consistent with the reduced inhibin production that has been described in the perimenopausal woman (13). It is also possible that this subject represents a subgroup of MODZT who have an inhibin deficiency unrelated to advanced reproductive age.

In summary, MODZT as a group do not have elevated gonadotropin levels to explain their propensity to double ovulate. When double ovulation occurs, this may be associated either with elevated ovarian steroids and inhibin with suppressed gonadotropin levels, suggesting an enhanced follicular sensitivity to gonadotropins, or elevated gonadotropins with reduced ovarian inhibin production. In monoovulatory cycles, MODZT have elevated follicular phase progesterone levels, which may reflect altered follicular steroidogenesis, reflecting, in turn, an altered intrafollicular environment that may favor follicular development over atresia.

### Acknowledgments

Special thanks go to our mothers of twins and control women who persevered with a demanding protocol, and to Marion Croft, our research nurse, whose tireless efforts made the study possible. Thanks also to the staff of Monash Ultrasound for Women and Sue Healy for assistance with recruitment and DNA fingerprinting. We are also grateful for the cooperation of the Australian Multiple Births Association. Clear-Plan test kits were generously supplied by Fisons Australia.

### References

1. Bulmer MG. 1970 The biology of twinning in man. Oxford: Clarendon Press.
2. Philippe P. 1985 Genetic epidemiology of twinning: a population based study. *Am J Med Genet*. 20:97-105.
3. Parisi P, Gatti M, Prinzi G, Caperna G. 1983 Familial incidence of twinning. *Nature*. 304:626-628.
4. Meulemans WJ, Lewis CM, Boomsma DI, et al. 1993 Segregation analysis of dizygotic twinning. *Am J Hum Genet*. 53(Suppl):A834.
5. Martin NG, Shanley S, Butt K. 1991 Excessive follicular recruitment and growth in mothers of spontaneous dizygotic twins. *Acta Genet Med Gamellol*. 40:291-301.
6. Nylander PPS. 1973 Serum levels of gonadotrophins in relation to multiple pregnancy in Nigeria. *J Obstet Gynaecol Br Commonw*. 80:651-653.
7. Nylander PPS. 1978 Causes of high frequency twinning in Nigeria. In: Nance WE, Allen G, Parisi P, eds. *Twin research. B. Biology and epidemiology*. New York: Liss; 55-60.
8. Martin NG, Olsen ME, Theile H, El Beaini JL, Handelsman D, Bhatnagar AS. 1984 Pituitary-ovarian function in mothers who have had two sets of dizygotic twins. *Fertil Steril*. 41:878-880.
9. Martin NG, Robertson DM, Chenevix-Trench G, de Kretser DM, Osbourne 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.
10. Chen P, Haywood N, Kidson C, Ellem KAO. 1990 Conditions for generating well-resolved human DNA fingerprints using M13 phage DNA. *Nucleic Acids Res*. 18:1065.
11. McLachlan RI, Robertson DM, Burger HG, de Kretser DM. 1986 The radioimmunoassay of bovine and human follicular fluid and serum inhibin. *Mol Cell Endocrinol*. 46:175-185.
12. Hurwitz A, Adashi EY. 1992 Ovarian follicular atresia as an apoptotic process: a paradigm for programmed cell death in endocrine tissues. *Mol Cell Endocrinol*. 84:19-23.
13. de Jong FH, Baird DT, van der Molen HJ. 1974 Ovarian secretion rates of estrogens, androgens and progesterone in normal women and in women with persisting ovarian follicles. *Acta Endocrinol (Copenh)*. 77:575-587.
14. Hodgen GD. 1986 Ovarian function for multiple follicular maturation. *Clin Obstet Gynecol*. 29:127-140.
15. Gougeon A, Testart J. 1990 Influence of human menopausal gonadotropin on the recruitment of human ovarian follicles. *Fertil Steril*. 54:848-852.
16. Hoff JD, Qigley ME, Yen SSC. 1983 Hormonal dynamics at mid-cycle: a reevaluation. *J Clin Endocrinol Metab*. 57:792.
17. Yong EL, Hillier SG, Turner M, Baird DT, Ng SC, Bongso A, Ratnam SS. 1994 Differential regulation of cholesterol side chain cleavage (P450scc) and aromatase (P450arom) enzyme mRNA expression by gonadotrophins and cyclic AMP in human granulosa cells. *J Mol Endocrinol*. 12:239-249.
18. Bieszczad RR, McClintock JS, Pepe GJ, Dimino MJ. 1982 Progesterone secretion by granulosa cells from different sized follicles of human ovaries after short term incubation. *J Clin Endocrinol Metab*. 55:181-184.
19. Chandrasekhar YA, Melner MH, Nagalla SR, Stouffer RL. 1994 Progesterone receptor, but not estradiol receptor, messenger ribonucleic acid is expressed in luteinizing granulosa cells and the corpus luteum in rhesus monkeys. *Endocrinology*. 135:307-314.
20. Loutradis D, Bletsis R, Aravantinos L, Kallianidis K, Michalas S, Psychoyos A. 1991 Pre-ovulatory effects of the progesterone antagonist mifepristone (RU486) in mice. *Hum Reprod*. 6:1238-1240.
21. Snyder BW, Beecham GD, Schane HP. 1984 Inhibition of ovulation in rats with epostane, an inhibitor of  $3\beta$ -hydroxysteroid dehydrogenase. *Proc Soc Exp Biol Med*. 176:238-242.
22. Zelinski-Wooten MB, Hess DL, Wolf DP, Stouffer RL. 1994 Steroid reduction during ovarian stimulation impairs oocyte fertilization, but not folliculogenesis, in rhesus monkeys. *Fertil Steril*. 61:1147-1155.
23. Trudeau VL, Matzuk MM, Hache RJ, Renaud LP. 1994 Overexpression of activin beta A-subunit mRNA is associated with decreased activin type II receptor mRNA levels in the testes of alpha-inhibin deficient mice. *Biochem Biophys Res Commun*. 203:105-112.
24. Findlay JK. 1993 An update on the roles of inhibin, activin and follistatin as local regulators of folliculogenesis. *Biol Reprod*. 48:15-23.
25. Hee J, MacNaughton J, Bangah M, Burger HG. 1993 Perimenopausal patterns of gonadotrophins, immunoactive inhibin, oestradiol and progesterone. *Maturitas*. 18:9-20.