Hormonal Contributions to the Recruitment of Follicular Development Following Ovarian Wedge Resection in Polycystic Ovary Syndrome

SHIGEO ARAKI, KOSHIRO CHIKAZAWA
AKIO AKABORI, KUNIHIKO IJIMA
AND TARO TAMADA

Department of Obstetrics and Gynecology, Jichi Medical School
Minamikawachi-machi, Tochigi, 329-04

Abstract

The present study was performed in order to elucidate the mechanism of the recruitment of follicular growth and subsequent ovulation after ovarian wedge resection (WR). Seven patients with a diagnosis of PCO underwent WR. After taking 4 or 5 preoperative blood samples in the morning after their hospitalization, blood was obtained following the operation at 3-h intervals for the first 48 h and at 1–3 day intervals thereafter for 9–33 days. These samples were analyzed for LH, FSH, estradiol, progesterone and androstenedione. Each ovarian tissue obtained by the operation was processed for histological examination. In 5 patients, the ovaries appeared to correspond with typical PCO. In the remaining 2 patients, the histological features of the ovaries were consistent with a type of sclero-cystico-atrophic ovary according to Kusuda (1979). Some characteristic postoperative hormonal changes were observed in the patients with typical PCO who ovulated postoperatively. FSH started to increase from 3 to 30 h after WR, and remained at higher levels for 2–7 days. LH increased in a sporadic form shortly after WR and then declined in spite of sustained higher levels of FSH. Preoperative levels of androstenedione in the patients with typical PCO ranged between 2.0–3.4 ng/ml, which exceeded serum levels in normal women. These high levels of androstenedione fell gradually following WR. There was an apparent decline of estradiol after WR in 2 out of 5 patients who ovulated postoperatively. A minor increase in progesterone occurred shortly after the operation, followed by a prompt decrease. In contrast, postoperative hormonal changes were minimal in the patients who did not ovulate postoperatively.

The postoperative hormonal changes including a decline in androstenedione, a temporary-minor increase in progesterone, a short-term sporadic increase in LH and a sustained increase in FSH may be involved in the mechanisms which account for the recruitment of follicular growth and subsequent ovulation after WR.

It has been shown that ovarian wedge resection (WR) frequently results in ovulation in patients with polycystic ovary syndrome (PCO) (Stein and Leventhal, 1935: Ingersoll and McDermott, Jr., 1950: Leventhal and Cohen 1951: Goldzieher and Axelrod, 1963). Although intensive studies have been performed to examine the hormonal changes which occur following WR, the mechanism of the restoration of ovulation after the operation remains to be fully elucidated. Some explanations have been proposed for the mechanism of ovulation following the surgical management. For years it has been believed that the removal of the mechanical barrier results in ovulation (Stein and Leven-
thal, 1935: Bailey, 1937: Ingersoll and McDermott, Jr., 1950). However, this hypothesis does not explain the fact that clomiphene citrate and human menopausal gonadotropin are able to induce ovulation in the majority of patients with PCO (Crooke et al., 1963: Whitelaw et al., 1964: Wang and Gemzell, 1980).

On the other hand, it has been suggested that hormonal alterations following WR are responsible for follicular maturation and subsequent ovulation (Stein and Leventhal, 1935: Stein and Cohen 1939: Ingersoll and McDermott, Jr., 1950). Earlier studies have revealed some characteristic hormonal changes including a postoperative decrease in testosterone (Forchielle et al., 1963: Lloyd et al., 1966: Judd et al., 1971) and androstenedione (Judd et al., 1971, 1976). Based on postoperative changes in gonadotropin and gonadal steroids, Judd et al., (1976) and Mahesh et al., (1978) insist that the mechanism responsible for ovulation following WR might be local rather than central since the changes in ovarian hormone secretion had no discernible effect on circulatory gonadotropin levels prior to a preovulatory surge.

Our recent studies have focused on the mechanism of the recruitment of the dominant follicle which is destined to ovulate in women (Araki, 1979). Our data suggested that increased secretion of FSH sustained for several days is a key event in the selection of a dominant follicle in a normal cycle or under some experimental conditions (Araki, 1979: Araki et al., in preparation for publication).

The present study was performed in order to reevaluate the early effects of WR on circulatory gonadotropin with reference to the recruitment of a dominant follicle.

**Materials and Methods**

Seven patients undergoing WR volunteered for the present study. Clinical data are summarized in Table 1. Two patients complained of obesity. None of these patients exhibited hirsutism. Preoperative hormonal studies showed that serum levels of estradiol were comparable to those seen in the midfollicular phase and the responses of LH to LH-RH were increased. LH levels were significantly higher in 5 out of 7 patients and the remaining 2 were normal in comparison with the basal levels observed in normal women. Vaginal examination revealed the existence of enlarged ovaries in all these patients. These clinical data suggested that the patients belong to the category of PCO (Nakamura et al., 1975; Devane et al., 1975; Patton et al., 1975; Kusuda, 1979). All patients were admitted to the hospital and underwent WR by which approximately one-third of each ovary was removed. The basal body temperature (BBT) was recorded before and after the operation.

Preoperative daily blood samples were obtained in the morning after hospitalization. An intravenous cannula with a three-way stopcock was inserted into the brachial vein shortly prior to the operation for frequent blood collection. After taking a control sample at the beginning of the operation, blood was obtained at 3-h intervals for the first 48h and 1-3 day intervals thereafter for 9-33 postoperative days. Some additional samples were obtained on occasion in patients who did not ovulate within consecutive blood collection periods.

Serum FSH, LH, estradiol and progesterone were measured by radioimmunoassay of which details were reported previously (Araki et al., 1978 a, b). Radioimmunoassay of plasma androstenedione was performed using the method which was described by Yoshida et al. (1974). All blood samples were analyzed for LH, FSH, estradiol and progesterone. Androstenedione was measured in the selected sample control before WR and the samples obtained for 48h postoperatively.

Each ovarian tissue obtained by the operation was processed for histological examination. The tissue embedded in paraffin was sectioned serially. Every eighth section was mounted and stained with hematoxin-l-eosin stain. Each section being 5 μ in width, there was a 30 μ space between each two sections examined. The sections were studied under a microscope equipped with a scale.

**Results**

**Ovarian histology**

In 5 patients (Cases #1～5), the enlarged ovaries were composed of multiple follicular cysts, stromal hyperplasia, and fibrous ovarian capsules. These findings appeared to correspond with typical PCO observed by others (Evans and Riley, 1958: Nishimura et al., 1970: Patton et al., 1975: Suzuki, 1976:
### Table 1: Clinical Data on 7 Patients Undergoing Wedge Resection

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>AGE</th>
<th>PREVIOUS PREGNANCY</th>
<th>WEIGHT (kg)</th>
<th>HEIGHT (cm)</th>
<th>MENSES</th>
<th>HIRSUTISM</th>
<th>PREOPERATIVE LEVELS OF CIRCULATORY HORMONES *</th>
<th>LH RESPONSE TO LH-RH</th>
<th>OVARY (COIFFED BY LAPAROTOMY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. K T</td>
<td>27</td>
<td>0</td>
<td>65</td>
<td>148</td>
<td>Olig</td>
<td>-</td>
<td>49.9</td>
<td>0.47</td>
<td>INCREASED ENLARGED PCO</td>
</tr>
<tr>
<td>2. H Y</td>
<td>26</td>
<td>0</td>
<td>65</td>
<td>150</td>
<td>Olig</td>
<td>-</td>
<td>85.4</td>
<td>0.55</td>
<td>INCREASED ENLARGED PCO</td>
</tr>
<tr>
<td>3. M S</td>
<td>26</td>
<td>0</td>
<td>56</td>
<td>153</td>
<td>Olig</td>
<td>-</td>
<td>75.0</td>
<td>0.73</td>
<td>INCREASED ENLARGED PCO</td>
</tr>
<tr>
<td>4. K K</td>
<td>25</td>
<td>0</td>
<td>45</td>
<td>154</td>
<td>Ame</td>
<td>-</td>
<td>82.0</td>
<td>0.52</td>
<td>INCREASED ENLARGED PCO</td>
</tr>
<tr>
<td>5. S T</td>
<td>27</td>
<td>0</td>
<td>52</td>
<td>150</td>
<td>Anov</td>
<td>-</td>
<td>65.0</td>
<td>0.31</td>
<td>INCREASED ENLARGED PCO</td>
</tr>
<tr>
<td>6. T H</td>
<td>26</td>
<td>0</td>
<td>48</td>
<td>148</td>
<td>Ame</td>
<td>-</td>
<td>78.3</td>
<td>0.68</td>
<td>INCREASED SLIGHTLY ENLARGED PCO</td>
</tr>
<tr>
<td>7. Y M</td>
<td>25</td>
<td>0</td>
<td>47</td>
<td>149</td>
<td>Ame</td>
<td>-</td>
<td>45.0</td>
<td>0.48</td>
<td>INCREASED SLIGHTLY ENLARGED PCO</td>
</tr>
</tbody>
</table>

Abbreviations: Olig = oligomenorrhea, Ame = Amenorrhea>3 > months, Anov = Anovulatory cycle, Ed = Estradiol, P = Progesterone, A = Androstenedione, LH = Luteinizing Hormone, FSH = Follicle Stimulating Hormone, PRL = Prolactin, PCO = Polycystic Ovary,

*Blood samples were obtained at the outpatient door within 1 month before ovarian wedge resection. The values were expressed in pg/ml for Ed, ng/ml for P, A and PRL, and mIU/ml for LH and FSH.

Hashiba, 1977: Kusuda, 1979). In this group, 3 patients had oligomenorrhea, another 1 secondary amenorrhea and the remaining 1 an anovulatory cycle.

In the 2 patients with secondary amenorrhea (# 6, 7) the ovaries were slightly smaller than those observed in the other group. Ovarian histology revealed enriched stroma, a decreased number of follicular elements with several microfollicular cysts and thick, fibrous capsules. These features were found to be consistent with a type of sclero-cystico-atrophic ovary (SCA) according to Kusuda (1979).

**Hormonal changes following WR**

Since hormonal levels following the operation varied from case to case, each set of individual results is illustrated in separate figures.

**Case 1:** Blood samples were obtained for 30 days after the operation. Hormonal patterns for 3 preoperative days and for the first 48 h after WR are illustrated in Fig. 1a. The preoperative concentration of LH was 23–33 mIU/ml. LH levels rose 3 h after WR and remained at higher levels (50–66 mIU/ml) for 12 h with only one exception observed at 6 h. Thereafter, plasma LH declined and fluctuated around the control level up to 48th postoperative h. The preoperative concentration of FSH was 13–14 mIU/ml. There was an apparent increase in FSH (21 mIU/ml) 9 h after WR, and the elevated levels (16–24 mIU/ml) were sustained until the end of the 48 h period of frequent samplings.

The preoperative level of androstenedione was 2.0–2.4 ng/ml, but this level promptly dropped in the 3 h period after WR. The lower levels of the steroid (0.5–1.3 ng/ml) were maintained with some fluctuations thereafter. Estradiol exhibited no remarkable change for 48 h following WR, remaining at a level of
around 100 pg/ml. The preoperative progesterone level was 0.5–0.7 ng/ml. It increased slightly for 6 h after the operation and then promptly decreased to around the preoperative level.

Daily hormonal patterns with a BBT curve are illustrated in Fig. 1b. A moderate increase in FSH was observed for 6 days after the operation, followed by a gradual decrease. No discernible changes in LH were seen until the 16th day after the operation on which a preovulatory surge of LH and FSH occurred. An increase in progesterone was seen following the occurrence of characteristic mid-cycle hormonal patterns which are similar to those observed at the mid-cycle of a normal menstrual cycle. These hormonal changes and a biphasic pattern in BBT gave proof of postoperative ovulation.

Case 2: Blood samples were obtained for 33 days after the operation. Hormonal patterns for 3 preoperative days and for the first 48 h after WR are illustrated in Fig. 2a. The preoperative concentration of LH was 22–33 mIU/ml. This level rose to 57 mIU/ml at 9 h, followed by a decrease at 15 h and thereafter fluctuated around the control level (24–48 mIU/ml). Levels in FSH increased gradually from 30 h after the operation.

The preoperative level of androstenedione was 3.1–3.7 ng/ml, but this level promptly fell during the 6 h after WR. The lower level of this steroid (1.2–2.1 ng/ml) was maintained thereafter. Estradiol showed no remarkable change for 48 h after WR. The preoperative progesterone level was 0.7–0.9 ng/ml. However, it increased to 3.2 ng/ml at 3 h, followed by a rapid decrease.

Daily hormonal patterns with a BBT curve are illustrated in Fig. 2b. A moderate
increase in FSH was seen 3 days after WR. This level fell gradually, reaching its nadir on the 17th postoperative day. LH was slightly higher for several days after the operation. A preovulatory gonadotropin surge was observed on the 18th and 19th days following a antecedent increase in estradiol. These midcycle hormonal changes were followed by a remarkable increase in progesterone. The hormonal changes and a biphasic pattern in BBT indicated a postoperative occurrence of ovulation.

Case 3: Blood samples were obtained for 21 days after the operation. Hormonal patterns for 3 preoperative days and for the first 48 h after WR are illustrated in Fig. 3a. Preoperative concentrations of LH and FSH were 25–48 mIU/ml and 7.4–9.0 mIU/ml, respectively. FSH levels started to increase at 18 h and remained at higher levels thereafter. The postoperative LH level fluctuated markedly with several spikes (49–65 mIU/ml), while the base level remained around the level of the preoperative concentration.

The preoperative level of androstenedione was 1.8–2.0 ng/ml. This level gradually fell during the 24 h after WR, followed by lower fluctuating levels (0.8–1.5 ng/ml) up to 48 h. Estradiol, also, decreased gradually form the preoperative level (80–118 pg/ml) to the lower level (39 pg/ml) by 15 h. Progesterone levels increased approximately two fold by 6 h, followed by a prompt decrease at 9 h and then remained at lower levels (0.4–0.6 ng/ml) up to 48 h after the operation.

Daily hormonal changes with a BBT curve are illustrated in Fig. 3b. FSH increased after surgery, remaining at the levels of 11–19 mIU/ml for several postoperative days. A gonadotropin surge was observed 20 days after WR following an antecedent increase in estradiol. Significant increases in progesterone were ob-

Fig. 2 and b. Serial steroid and gonadotropin levels with a BBT curve before and after ovarian wedge resection in a patient, HY, who ovulated postoperatively. Fig. a and b show hormonal changes in the blood samples obtained at 3-h intervals for the first 48 h and at 1–3 day intervals for 33 postoperative days, respectively.
Fig. 3 a and b. Serial steroid and gonadotropin levels with a BBT curve before and after ovarian wedge resection in a patient, MS, who ovulated postoperatively.

Fig. a and b show hormonal changes in the blood samples obtained at 3-h intervals for the first 48h and daily for 21 postoperative days, respectively.

observed on the 21st postoperative day. The BBT curve showed a biphasic pattern after surgery. The finding on the circulating hormones and BBT suggested a spontaneous ovulation following the operation.

Case 4: Blood samples were obtained for 11 days after WR. Hormonal patterns for 3 preoperative days and for the first 48 h after WR are illustrated in Fig. 4a. The preoperative level of LH was 28–37 mIU/ml. This level rose to 79 mIU/ml by 6 h after the operation and thereafter remained at higher levels with remarkable fluctuations (35–70 mIU/ml) up to 48 h. FSH levels rose gradually following WR, reaching 30 mIU/ml at the end of the 48 h period of frequent samplings.

The preoperative level of androstenedione was 2.1–2.3 ng/ml. A marked decrease in this steroid was observed at 12 h and thereafter the low levels were maintained up to 48 h with one exception at 18 h. A postoperative decrease in estradiol was seen for the first 12 h after WR. Progesterone increased significantly by 3 h, followed by a prompt decrease at 6 h and then the lower levels were maintained up to 48 h.

Daily hormonal changes with a BBT curve are illustrated in Fig. 4b. Significant increases in FSH were observed for several days after WR, declining to levels around the preoperative level thereafter. LH concentrations were high (64 and 72 mIU/ml) for 2 days after WR and then fell to close to the control level. Estradiol levels fluctuated between 50 and 140 pg/ml throughout 11 postoperative days. However, no remarkable changes in progesterone levels were observed in the same period. Additional blood samplings were made on the 16th, 22nd and 27th postoperative days on which BBT showed a high phase. The concentrations of progesterone in these blood samples were 8.1, 13.2 and 4.9 pg/ml. The daily hormonal changes and the biphasic pattern of BBT indicated that ovulation occurred approximately 2 weeks after the operation.

Case 5: Blood samples were obtained for 9 consecutive days after the operation, followed by 2 additional samplings taken on the 22nd and 26th postoperative days. Fig. 5a shows the hormonal patterns for 3 preoperative days and for the first 48 h after WR. The preoperative concentration of LH was 43–50 mIU/ml. This level rose to 206 and 210 mIU/ml at 3 and 6 h after the operation, and then declined rapidly. There was a 1.7-fold in-
Fig. 4 a and b. Serial steroid and gonadotropin levels with a BBT curve before and after ovarian wedge resection in a patient, KK, who ovulated postoperatively. Fig. a and b show hormonal changes in the blood samples obtained at 3-h intervals for the first 48 h and daily for 11 postoperative days. (See the text for hormonal levels in the additional samples).

Fig. 5 a and b. Serial steroid and gonadotropin levels with a BBT curve before and after ovarian wedge resection in a patient, ST, who ovulated postoperatively. Fig. a and b show hormonal changes in the blood samples obtained at 3-h intervals for the first 48 h and daily for 9 postoperative days. (See the text for hormonal levels in the additional samples).

crease in FSH at 3 h. The higher levels of FSH (23–32 mIU/ml) were maintained throughout the period of frequent samplings.

Androstenedione decreased gradually to approximately 50% of the preoperative level by 12 h after the operation and then fluctuated around the level of 1 ng/ml for 48 h. No remarkable changes in estradiol were observed after WR. Progesterone levels rose to 2–3 times as high as the preoperative level at 3 h and at 24–27 h after WR.

The daily hormonal changes with a BBT curve are illustrated in Fig. 5b. LH increased 2.7-fold on the 1st day after WR, followed by
a sudden decrease on the 2nd day. Moderate increases in FSH were observed for the first 3 days after WR. No gonadotropin surges were found by the 9th day after the operation. Progesterone in the samples obtained on the 22nd and 24th days were 12 and 9.0 ng/ml, respectively. The occurrence of postoperative ovulation was suggested by a biphasic pattern of BBT and by significant increases in progesterone.

Case 6: Only minor hormonal changes were observed in this case (Fig. 6). A significant increase in progesterone was seen at 3 h and it then declined to its preoperative level by 9 h. Androstenedione decreased significantly by 6 h and thereafter fluctuated around the lower level. Since no remarkable hormonal change in the daily samples was observed up to 4 weeks after WR, daily hormonal patterns are not illustrated. The BBT curve was an irregular pattern, as shown in Fig. 6. The postoperative hormonal changes and the BBT patterns in this subject indicated the absence of postoperative ovulation.

Case 7: There were only minor hormonal changes following the operation (Fig. 7). No discernible changes in gonadotropin levels were not found except a slight increase in LH at 3 h. Progesterone increased slightly at 3 and 6 h, followed by a decrease to the preoperative level by 12 h and then fluctuated within a small range. Preoperative concentrations of androstenedione were 1.1–1.8 ng/ml. These levels decreased by 12 h after
WR, and then remained at lower levels. Since daily hormonal levels exhibited only minor changes, the daily patterns of the circulating hormones are not illustrated. The BBT curve showed a monophasic pattern after the operation (Fig. 7). The postoperative hormonal changes and the pattern of BBT gave no indication of ovulation after WR.

Discussion

In the present study, 7 patients underwent WR for the induction of ovulation and for an ovarian biopsy. Since these patients did not ovulate on most occasions in response to clomiphene, surgical treatment was required. Five patients with a histology of typical PCO ovulated 2–3 weeks after WR. The remaining 2 patients, with a type of SCA, failed to ovulate.

The postoperative hormonal changes were minimal in the patients who did not ovulate. On the other hand, some characteristic changes in circulating gonadotropins and steroids were observed following the operation in the subjects with postoperative ovulation. Although WR affected peripheral levels of LH, FSH, progesterone and androstenedione, the magnitude of the responses varied markedly from case to case.

For the gonadotropins, postoperative increases in FSH were observed in 5 patients who ovulated. FSH started to increase from 3 to 30 h after WR, and remained at higher levels for 2–7 days. It is well known that in normal women there is an early "follicular phase" rise of circulating FSH which is probably necessary for the stimulation of follicular growth and subsequent ovulation (Ross et al., 1970). It is reasonable to consider that an increase in FSH observed after WR might contribute to the recruitment of follicular maturation in patients with PCO. The magnitude and duration of the postoperative FSH increase varied markedly among the patients. The potency of FSH stimulation required for the development of a dominant follicle must depend on the degree of preoperative follicular maturation.

Our results did not coincide with the findings reported by Judd et al. (1976) who found stable gonadotropin levels prior to ovulation following WR. Although this disagreement remains to be explained, it may represent the clinical differences in this syndrome which spread in a wide spectrum.

In the subjects with postoperative ovulation, LH increased in a sporadic form shortly after WR and then declined in spite of sustained higher levels of FSH. Our data support the results reported by Katz et al. (1978) who have observed significant postoperative changes in circulatory gonadotropin levels, resulting in a lowering of the LH: FSH ratio in 2 patients with PCO. In a previous study, we reported that LH levels increased following the administration of progesterone in women pretreated with a prolonged infusion of estradiol (Araki et al., 1978a). Chang and Jaffe (1978) reported that a minor increase in progesterone (appropriately 1 ng/ml) elicited LH increases in women pretreated with estradiol. In the present study, we observed prompt increases in serum progesterone which might stimulate LH release following WR.

It has been known that there is increased ovarian androgen production in patients with PCO (Southren et al., 1969; Bardin and Lipsett, 1967; De Vane et al., 1975: Kasuga, 1980). Preoperative levels of androstenedione in our patients with typical PCO ranged between 2.0–3.4 ng/ml, which exceeded serum levels (0.5–1.5 ng/ml) observed in normal women. These preoperative high levels of androstenedione fell gradually following the operation. A similar postoperative decrease in androstenedione has been reported by others (Judd et al., 1976; Mahesh et al., 1968).

For estradiol there was an apparent decline in estradiol following WR in 2 out of 5 patients who ovulated postoperatively. Since the steroid is derived predominantly from extraglandular aromatization of androgen (Siiteri
and MacDonald, 1973), a reduction in ovarian tissue by WR may not always bring on discernible changes in serum estrogen levels. In the subjects who appeared to ovulate within the period of daily samplings, a rise in estradiol was observed 16–19 days after WR followed by an increase in progesterone. These patterns in gonadal steroids were comparable to those observed at the mid-cycle in normal women.

WR probably decreases the secretion of ovarian steroids by reducing the number of hormone producing sites. Major surgical stress may influence the secretion of steroid hormones by means of postoperative changes in gonadotropin levels (Aono et al., 1976). Surgical manipulation of the ovaries may enhance the release of the steroids by squeezing and disrupting the ovarian tissue.

For the androgens, a postoperative reduction in androstenedione may represent a decreased secretion of the steroid from the adrenal under surgical stress. However, a decrease in androstenedione is more marked and more persistent after WR in patients with PCO in comparison with that observed following hysterectomy in patients with non-ovarian disease (Judd et al., 1976). Significant decreases in urinary 17-ketosteroid are more commonly observed following WR rather than ovarian incision (Tanaka et al., 1978). On the basis of these data, it is reasonable to consider that a reduction in androstenedione is attributable mainly to the specific effects of WR.

Some possible mechanisms of follicular development and subsequent ovulation after WR have been proposed. First, the operation may remove a mechanical barrier which accounts for the impairment of follicular development and ovulation (Stein and Leventhal, 1935: Bailey, 1937: Ingersoll and McDermott, Jr., 1950). Secondly, the operation may cause local inflammatory changes (i.e. hyperemia, vasodilation) which result in increased exposure of the ovarian tissue to circulatory gonadotropins (Judd et al., 1976). Thirdly, a drop in basal LH levels after WR may be preferable for the resumption of follicular development (Tanaka et al., 1980). In addition to these explanations we would like to emphasize the possibility that the recruitment of follicular growth may be caused by the increase in FSH which is observed shortly after WR.

Although the present data demonstrated that WR in the patients with typical PCO induced circulatory hormonal changes which include a decline in androstenedione, a temporary minor increase in progesterone and a short-term sporadic elevation of LH and a sustained increase in FSH, further in-depth studies on larger numbers of patients will be necessary to elucidate a definite relationship between postoperative hormonal changes and the mechanism of ovulation following the operation.

**Acknowledgements**

We are grateful to Teikokuzohki Central Laboratory for the help with the radioimmunoassay of androstenedione. We also thank Miss Y. Hosokawa for her excellent secretarial assistance, and Misses T. Takashio and M. Yamai for their stillfull technical assistance.

**References**


Bailey, K. V. (1937). The operation of extroversion of the ovaries for functional amenorrhea especially


Wang, C. F. and C. Gemzell (1980). The use of hu-
man gonadotropins for the induction of ovulation
in women with polycystic ovarian disease. *Fertil.
Steril.* 33, 479–486.
Whitelaw, R. B., L. R. Gramselland, W. H. Stamm
(1964). Clomiphene citrate, its uses and observa-
90, 355–363.
Yoshida, T., K. Tanemori, K. Inatomi, Y. Ozaki, S.
Radioimmunoassay of steroid hormone (6)—Radio-
immunoassay of Δ-4-androstenedione. Hormone to