ON THE ENDOCRINE FUNCTION OF THE HUMAN GRAAFIAN FOLLICLE

By

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A thesis submitted to the University of Adelaide
in fulfilment of the requirements
for the degree of
Doctor of Medicine

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December, 1977

I N D E X

4. A HISTOCHEMICAL ASSESSMENT OF THE SITE AND ACTIVITY OF THE ENZYME Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE (3β-HSD) IN RELATION TO THE STEROID ACTIVITY OF HUMAN GRAAFIAN FOLLICLES IN ORGAN CULTURE, EXPLANTED THROUGHOUT THE MENSTRUAL CYCLE AND IN THE IMMEDIATE POST PARTUM PERIOD: COMPARISONS BETWEEN FOLLICLES INCUBATED WITH AND WITHOUT GONADOTROPHINS

SUMMARY

Two hundred and eight human Graafian follicles with diameters ranging from 3-12 millimetres were obtained from eighty nine ovarian biopsies, which were performed in women who had spontaneous, regular menstrual cycles. These follicles were incubated either as whole follicles in organ culture or dissected into their respective theca and granulosa cell components and incubated separately. In some instances gonadotrophins were included in the culture medium. The culture medium was aspirated daily and its content of testosterone, oestradiol-17β and progesterone estimated by validated radioimmunoassay procedures. The follicles were either processed histologically for assessment of cellular morphology, theca and granulosa layer thickness and cell size, or were examined histochemically in an attempt to localize the site and activity of the enzyme, Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD), at various times throughout the incubation period.

The following conclusions were drawn:-

1. The maintenance of isolated human follicles in organ culture to facilitate useful endocrine investigations has been achieved. Observations indicated that follicular growth was a continuum, but that rapid follicular growth occurred only during the late follicular phase of the reproductive cycle. In culture, endocrine function as assessed by steroid release was sustained but varied between individual follicles. This variation appeared related both to follicular size and the stage in the menstrual cycle when follicles were removed.

2. Follicles removed during the late follicular phase (preovular) of the cycle were significantly larger than follicles removed during the early follicular phase, luteal phase or the immediate post partum period. No significant difference in size could be demonstrated in follicles removed during the early follicular or luteal phase of the menstrual cycle. Follicles removed during the early post partum period were significantly smaller than both the luteal and the early follicular phase follicles.

3. On a weight for weight basis steroid activity in late follicular phase follicles appeared to be related to the size of the follicle. The release of oestrogen and progesterone increased progressively with increasing follicular size and approaching ovulation. A steady rise in testosterone release occurred until the late follicular phase follicle

reached a diameter of 8 mm, and then declined, coincident with a progressive increase in oestrogen and progesterone release. This change in steroid activity coincided with the appearance of pronounced 3β-HSD activity in the granulosa cell layer but with persistance of 3β-HSD activity in the theca layer.

4. Invitro, the smaller early follicular phase follicles released testosterone predominantly, and 3β-HSD activity mainly was confined to the theca interna cells. Luteal phase follicles also released similar amounts of androgen but less amounts of oestrogen and progesterone than late follicular phase follicles and retained moderate activity of 3β-HSD in both theca and granulosa cells. Follicles removed within the first week of parturition maintained steroid activity, which was comparable with that of unstimulated late follicular phase follicles, and also possessed moderate 3β-HSD activity in both the theca and granulosa cells.

5. Invitro, the steroid release by isolates of theca and granulosa cells obtained from late follicular phase follicles, indicated that the theca was the primary source of testosterone synthesis and the granulosa cells the primary source of progesterone synthesis. Interestingly, isolates of both theca and granulosa cells were able to produce oestrogen, indicating that both cell types may contribute to oestrogen synthesis within the follicle, thereby lending support to the two cell theory for optimal oestrogen production.

6. Small slices of postmenopausal ovary which were devoid of follicles released small amounts of testosterone and progesterone but no oestradiol-17β. Histochemical localization for 3β-HSD in these ovaries demonstrated small patches of positive reaction indicating focal areas of steroid activity.

7. The incubation of follicles with gonadotrophins indicated that follicular phase follicles develop an ability to respond to FSH for both oestrogen and progesterone production, but that the luteal phase follicles tended to maintain their capacity to respond to FSH for increased progesterone production (a reflection of granulosa cell activity) but not for increased oestrogen production (a possible indication of failing theca cell aromatase activity). Incubation with HCG was associated with an increased progesterone output in both follicular and luteal phase follicles, increased testosterone output by follicular phase follicles but at the same time a decrease in the oestrogen production

by these follicles. This observation was consistent with the hypothesis that HCG may inhibit aromatase activity in the follicular phase follicle. The luteal follicle theca cells lost their ability to produce an increased release of oestrogen and testosterone following incubation with either FSH or HCG, however morphologically they did not regress as do the theca cells of the corpus luteum. Although post partum follicles produced steroids, invitro, they failed to respond to FSH apart from a transient increased output of progesterone. This observation was consistent with the hypothesis that FSH receptor sites were either sparse or non functional in these follicles.

8. Preliminary observations suggested that the addition of high concentrations of prolactin to the incubation media of whole follicles did not augment steroidogenesis and may well have an inhibitory effect, even in the presence of FSH.

9. Preliminary observations also suggested that human ovarian follicles were able to release relaxin, invitro. Maximum release of relaxin occurred from those follicles explanted during the luteal phase and in particular from the follicles removed from the ovary containing the corpus luteum. This hormone may be involved in the corpus luteum and in follicle regression or as a mediator of collagenase activity.

10. Collagen was demonstrated in the theca interna but was more marked in the theca externa of follicles from 2 to 12 mm in diameter. Collagen deposition was more abundant as the follicles became larger and was also present in luteal phase follicles. Collagen deposition was dispersed in the theca cells of the corpus luteum but was present in abundance in the surrounding stroma. The basement membrane lying between the theca and granulosa cells of follicles was prominent and stained identically to that of collagen. Evidence of the basement membrane or its remnants were not seen in corpora lutea. The circumferential pattern of collagen surrounding follicles probably appeared more prominent as the follicles grew due to a condensation effect on the surrounding connective tissue.

11. The combination of anaesthesia and surgery was associated with a significant elevation of blood prolactin levels. However follicular fluid prolactin levels obtained during surgery appeared to be independent of blood levels over the time interval studied.