Alteration in circulating LH level and in cervical mucus induced by mid-cycle progesterone in chimpanzees

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Summary. Administration of 200 µg mestranol/day (oestrogen) to ovariectomized chimpanzees caused a rapid decrease of circulating gonadotrophin concentrations to values similar to those in intact females. Administration of 2 mg chlormadinone acetate (progestagen) resulted in a prompt and significant rise in LH and FSH. This rise was accompanied by alteration in the physical characteristics and electrolyte composition of the cervical mucus which were the same as those observed around the time of ovulation. These results suggest a role of preovulatory progesterone secretion in relation to changes associated with ovulation in the chimpanzee.

Introduction

Studies over the past decade have demonstrated that the hormonal events that occur during the menstrual cycle of the chimpanzee closely parallel those seen in women (Graham, Collins, Robinson & Preedy, 1972; Graham, Keeling, Chapman, Cummins & Haynie, 1973; Reyes, Winter, Faiman & Hobson, 1975; Graham, 1981). The anatomy of the reproductive tract, including the cervix, is very similar to that of women (Wislocki, 1932; Gould & Martin, 1981) and follicular development and ovarian ovulatory physiology also appear to be similar (Graham & Bradley, 1972; Ansari & Gould, 1980). Previous work on chimpanzees and man has shown that progesterone levels rise before ovulation, coincident with the initiation of the ovulatory LH surge. The role, if any, of this hormone in ovulation has been obscure. Since the early 1970s, many publications have appeared which implicate follicular oestrogen secretion with induction of LH secretion by influencing both hypothalamic and pituitary function (e.g. Karsch et al., 1973; Vande Wiele & Ferin, 1979; Weick & Pomerantz, 1979; Garcia, Jones & Wright, 1981). Such reports have focussed on oestrogen alone and have not deliberately investigated any role of progestagen(s) in the system.

To investigate further the relationship of circulating steroids with ovulation and with cervical mucus composition, this study was directed towards identifying the effect of progestagen induction of the preovulatory surge of LH. In addition, we wished to evaluate alteration of cervical mucus electrolytes during this period because initial studies of chimpanzees and women suggested certain correlations between circulating hormone levels and electrolyte composition of the cervical mucus (Gould, 1978; Gould & Ansari, 1981).

In women, oestrogen treatment alters the physical properties of cervical mucus in a manner similar to that seen at mid-cycle (Jacobelli, Carcea & Angeloni, 1979). In general, progesterone counteracts the effect of oestrogen, e.g. pituitary sensitivity is reduced, cervical mucus is more viscous and less crystallized, and the sexual swelling of the chimpanzee is reduced in size. Alteration in physical properties of cervical mucus was documented in association with use of oral contraceptives containing progestagen and oestrogen (Singh & Boss, 1973). Such changes in the
physical properties and composition of cervical mucus are very similar to those observed in the luteal phase of the menstrual cycle and during pregnancy. Previously reported work has provided a description of the changes in visco-elasticity and electrolyte composition of cervical mucus during the menstrual cycle of the chimpanzee and human using the techniques applied here (Gould, Martin & Graham, 1976; Gould & Ansari, 1983).

### Materials and Methods

The subjects of this study were 3 female chimpanzees that had been ovariectomized 8 years before the start of these experiments, and had received periodic oestrogenic or progestational stimulation in connection with other studies. In the present study, these animals were treated with exogenous hormones according to four protocols (Text-fig. 1). Each protocol was repeated on two occasions separated by a period of 4–6 weeks to allow circulating gonadotrophin levels to rise to a level characteristic of the ovariectomized female. The basic stimulation protocol comprised 200 µg mestranol (Sigma, St Louis, MO., U.S.A.) by mouth for 14 days with the progestational agent, chlormadinone acetate (4 mg per day by mouth (Sigma Lot No. B3)), for 13 days in an overlapping sequence for 5 days as shown in Text-fig. 1(a). This represents the basic protocol for mimicking the normal menstrual cycle in these animals without, however, producing the normal oestrogen rise in the luteal phase.

![Diagram of hormone administration protocols](Text-fig. 1)

**Text-fig. 1.** Diagrammatic representation of the protocols for hormone administration. M = 200 µg mestranol by mouth per day; M2 = 600 µg mestranol by mouth per day; CA = 4 mg chlormadinone acetate by mouth per day. Protocol A is the treatment that mimics the natural cycle. Protocols B, C and D represent hormone administration patterns used to eliminate the effect of prolonged (b) or increasing (c) oestrogen levels, and of the influence of progestagen after increased oestrogen (d).

The remaining protocols (Text-figs 1b, c & d) represent modifications of the initial protocol to ensure that prolonged oestrogen stimulation alone (Text-fig. 1b), or increasing oestrogen levels (Text-fig. 1), were not responsible for the effects observed with progestagen treatment. The protocol in Text-fig. 1(d) was used to provide preliminary data on whether the effect of progestagen would be maintained even in the face of elevated oestrogen levels.

Gonadotrophins (LH, FSH) were assayed using published methods and antisera directed to human hormones (Graham, Gould, Collins & Preedy, 1979). FSH and LH were measured by double-antibody RIAs using the NIAMDD kit. The RIA for FSH used human FSH antibody No. 3
at a final dilution of 1:30 000 as the first antibody and radioiodinated human FSH (LER-1801-3) as the trace. The RIA for LH used human LH antibody No. 2 at a final dilution of 1:200 000 as the first antibody and radioiodinated human LH (LER-960) as the trace. The second antibody, ovine anti-rabbit γ-globulin, was used at a final dilution of 1:100. The human gonadotrophin standard (LER-907) used for both gonadotrophins showed parallelism with serial dilutions of chimpanzee plasma, as has been previously reported when human reagents are used to estimate the concentration of chimpanzee gonadotrophins (Howland, Faiman & Butler, 1971). One milliunit of chimpanzee FSH is the radioimmunoassayable activity equivalent to 50 µg LER-907; 1 mU chimpanzee LH is equivalent to 16-66 µg LER-907. Within-assay variability was 5%; between-assay variation was 15%. The least detectable amount of gonadotrophin in the duplicate 200-µl samples assayed was 0·2 mU for LH and 0·1 mU for FSH. Satisfactory assays were not available for the circulating forms of the synthetic steroids used, but the treatment doses (200 µg mestranol and 2 mg clomipramine) were those determined and used previously for inducing external signs of menstrual cyclicity, including full development of the perineal swelling and menstrual bleeding, after an interval from the end of oestrogen stimulation which was identical to that observed in intact adult females in our colony (K. G. Gould, unpublished data). These doses were, therefore, considered to be effectively physiological.

Blood and cervical mucus were obtained under ketamine HCl anaesthesia (8 mg/kg body weight) at 48-h intervals from animals treated with hormones as in Text-fig. 1. Blood was obtained by femoral venepuncture and cervical mucus was collected by direct aspiration into a preweighed, desiccated conical polyvinyl chloride tube. Cervical mucus production in the chimpanzee is not as copious as in women, and mucus was collected only from the distal portion of the cervical canal. An aliquot of cervical mucus was immediately placed on a carbon chip for X-ray analysis of elemental composition on an ISI Super III SEM (Santa Clara, CA 95050, U.S.A.) and a Tracor Northern NS880 X-ray analyser coupled to a nuclear semiconductor detector (Middleton, WI 53562, U.S.A.). The elemental composition of the collected cervical mucus was recorded as X-ray counts collected in a 50 sec period, and the ratios of elements were calculated directly from X-ray counts without scaling or correction for detector efficiency. This method has been described in detail elsewhere (Gould, 1978).

Visco-elasticity (spinnbarkeit) of the mucus was measured in mm, and the net weight of the remaining cervical mucus was determined using a Cahn electro-balance. The dry weight and then the percentage of water in the cervical mucus was determined after desiccation for 48 h to constant weight. Values for hormone concentration, electrolyte concentration and spinnbarkeit were compared for each group of 3 values derived from different animals using Student's t test, and tested for significance at the 95% confidence level (P < 0·05). Comparisons were made for each of 2 groups of data as the animals were used on two occasions.

Results

At the start of the experimental treatments, gonadotrophin levels were elevated significantly in all 3 animals (LH 33–39 mIU/ml, FSH 75–115 mIU/ml above normal circulating levels (Gould & Ansari, 1981) and the FSH/LH ratio was 1–3:1.

Administration of oestrogen resulted in prompt reduction in FSH and LH values to levels similar to those seen in normally cyclic animals; these concentrations were maintained during the first 8–9 days of oestrogen administration (Text-figs 2a & 2b) (Graham, 1981). Within 24 h after administration of clomipradon acetate, there was a significant elevation of LH levels. On one occasion, the concentration was nearly the same as that before administration of oestrogen. FSH was also significantly elevated, although not to the same degree (Text-figs 2a & b). Significant differences existed between the samples before and after administration of clomipradon acetate, and between the same sample in test and control cycles.
Text-fig. 2. Alteration of circulating levels of (a) LH and (b) FSH in the chimpanzee treated with hormones as indicated in Text-fig. 1(a). Values represent the mean for 6 observations plus the range.

This prompt rise in LH and FSH after administration of chlormadinone acetate was a consistent finding in these experiments. The time course and magnitude of the rises were within the range observed for chimpanzees ovulating normally (Gould & Ansari, 1981) in 4 of the 6 experiments, and greater in the other two. It was not observed in animals maintained on oestrogen alone for 16–18 days, or after administration of 600 µg mestranol/day in animals initially given 200 µg mestranol/day. When using Treatment D, a moderate (250% LH: 23–27 m.i.u./ml) rise in LH concentrations occurred, but the elevation was delayed by 24 h.

Elevation of potassium levels was noted in all samples within 48 h of the start of mestranol treatment, although this was not statistically significant. Potassium levels were lower during the rest of the experiment (Text-fig. 3a). Sodium values fell throughout the experiment (Text-fig. 3b). The initial administration of chlormadinone acetate resulted in a significant increase in the Cl : Na + K ratio (Text-fig. 3c).

During oestrogen treatment, spinnbarkeit of the cervical mucus increased significantly (10–15 fold) with maximum levels being obtained 23–48 h after the start of progesterone administration, i.e. around the time of ovulation in an intact animal (Text-fig. 3d).
Text-fig. 3. Alteration of (a) potassium and (b) sodium concentration, (c) the ratio of chloride to total sodium and potassium in, and (d) spinnbarkeit of cervical mucus of chimpanzees treated with hormones as indicated in Text-fig. 1(a). Values are the mean for 6 observations plus the range.

Discussion

The results of these experiments show that, although there is a progression of events occurring in response to stimulation with exogenous oestrogen alone that approach the conditions seen around the time of ovulation, major changes in hormone levels and cervical mucus characteristics do not occur until several hours after progestagen treatment is also given.

The alteration in endogenous hormone levels after mestranol treatment initially followed a predictable pattern. In the ovariectomized female, gonadotrophin levels are markedly elevated. These concentrations were reduced to normal cycle values by oestrogen administration in all the chimpanzees studied. Results of elemental analysis and physical characterization of cervical mucus substantiated the hypothesis that low levels of oestrogen favour secretion of potassium into the cervical mucus (Gould et al., 1976). The ratio of Cl: Na + K was previously shown to vary throughout the menstrual cycle, and to peak around the time of ovulation (Gould & Ansari, 1983) and this change was observed in this study. The percentage of water in the cervical mucus was not influenced as significantly by oestrogen stimulation as would have been expected from data in the literature derived from normally cyclic females (Kopito, Cosasky, Sturgis, Lieberman & Shwachman, 1973).

The clarity of the response of LH secretion to chlormadinone acetate treatment was unexpected. Initially, it was considered to result either from conversion of progestagen to oestrogen or from an effect of oestrogen which did not occur until rising oestrogen levels had been present for a period of several days, approximating the duration of oestrogen exposure in our initial experiments. These possibilities were effectively excluded by continuing oestrogen administration at the original dose level for up to 4 times the initial period and by giving oestrogen for the initial period and then increasing the oestrogen dose.

These results suggest a role of progestagen in initiation of the preovulatory LH surge. The source of the progestagen observed during natural cycles in intact animals is presumed to be cells of...
the ovarian follicle. This interpretation would suggest a requirement for some factor stimulating progestagen synthesis by follicle cells to be present before progestagen secretion. This, in turn, indicates that the early progestagen output in intact animals serves to potentiate, rather than initiate, LH output. It is not certain that the natural progestagen is progesterone per se, although progestagen levels do appear to rise before ovulation. In women, the time of the preovulatory LH surge may be better predicted by measurement of total oestrogen and an oestrogen:progestagen ratio than by assay of total oestrogen alone (Garcia et al., 1981). The present study is the first to focus specifically on the effect of progestagen in oestrogen-primed females of a non-human primate, and provides some support for the use of the oestrogen:progestagen ratio as an indicator of ovulation time in women.

Progestagen enhances maturation of rhesus monkey oocytes in vitro (Gould & Graham, 1976). The modest increase in circulating progesterone demonstrated to occur before ovulation, if derived from components of the follicle, would represent a significant concentration in the immediate vicinity of the maturing oocyte, indicating a further role for preovulatory progestagen.

The model used in this study is artificial in so far as ovariectomized females were used and orally-active hormones were administered. We believe the model represents an acceptable picture of the changes occurring around ovulation, however, because of the normal physiological changes in perineal skin and cervical mucus which were recorded, demonstrating a maintenance of end organ response, presumably as a result of the repeated exposure of the animals to gonadotrophins during the period after ovariectomy (see 'Materials and Methods'). The evidence presented here, together with other data on oocyte maturation (Gould et al., 1976), demonstrates a more important role of preovulatory progestagen in induction of normal preovulatory changes than heretofore ascribed.

References


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