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Reproductive hormone patterns in the female guinea-pig serum during the estrous cycle

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Introduction

Among laboratory animals the female guinea-pig estrous cycle shows many similarities with the human female reproductive cycle. Thus, the guinea-pig has an estrous cycle of 16 days, which includes spontaneous regular ovulation (defined as day 1) (*Stockard & Papanicolaou* 1917, 1919, *Ishii* 1928, *Young* 1936/37, *Burgos & Wislocki* 1958, 1965). If conception occurs the ovum reaches the cavum uteri at the end of day 3 (*Bishoff* 1852, *Squier* 1932). Implantation occurs at cycle day 6–7 (*Bishoff* 1852, *Spee* 1901).

A rough characterization of the estrous cycle can be made from inspection of the vaginal membrane. Thus the vagina is open at the time around ovulation as confirmed by smear stainings (*Stockard & Papanicolaou* 1917). Several attempts to characterize the circulating hormones during the guinea-pig reproductive cycle have been made during the last decades. Thus, it has been shown that progesterone levels are low around ovulation and increase from cycle day 2 to 5 (*Challis et al.* 1971) and reach a maximum at cycle day 6 to 9 after which time decreasing values are obtained. Serum estrogen levels have been more difficult to estimate, probably because of very low serum levels (*Croix & Franchimont* 1975). The study of *Garris & Mitchell* (1979) included results on serum estradiol-17β levels indicating a preovulatory peak and another increase at the time for ovum implantation. An LH-peak preceding ovulation has been shown, and curiously a postovulatory rise in FSH (*Croix & Franchimont* 1975). No information is available on prolactin levels in the guinea-pig.

The cited reports were all based on single blood samples collected from individual animals either by heart puncture during anaesthesia or by killing the animal. The results have been presented as mean group values for cycle days evaluated by vaginal smears. The aim of the present study was to follow the cyclic pattern of estradiol, progesterone, IR-LH, IR-FSH and IR-prolactin serum levels in individual animals during consecutive days in the estrous cycle.

Materials and Methods

Animals

Eleven sexually mature mottled or albino virgin female guinea-pigs (400–500 g) and two males all of English short hair strain were purchased from a local breeder. The animals were given pellets and water ad libitum and caged together 4 to 5. Lights were on from 08.00 a.m. to 08.00 p.m.

Estrous cycle determination

The animals were controlled daily concerning the status of the vaginal closure membrane. All animals showed regular cycles (n = 11). After at least two normal cycles the animals entered the study.

Surgical procedure

Two animals were subjected to bilateral ovariectomy which was performed via dorsal incisions under sodium pentobarbital anaesthesia one week before blood sampling was started (*Hammarström* 1980).

Pregnancy

Three animals were caged with one male and pregnancy was calculated from the second day of vaginal opening (*Sisk* 1976). These animals were used for prolactin determination.

Blood samples

At a dorsal lateral point of the lower limb of the guinea-pig, vena saphena lateralis could be identified after shaving. Vein puncture was carried out between 9.00 and 10.00 p.m. The blood (0.2–1.0 ml) was left for two hours at 2–5°C and then centrifuged at 800 × g. The serum was frozen at −20°C until assays were performed.

Hormone assay

Immunoreactive luteinizing (IR-LH), follicle stimulating (IR-FSH), hormones and IR-prolactin levels were determined in serum with assays systems primarily developed for investigation in the rat (kits from the National Hormone and Pituitary program, the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institute of Health, Bethesda, USA) as described previously (*Andersson et al.* 1988). 50 µl of serum was used for each analysis.

Estradiol-17β or progesterone was determined with radioimmunoassay (RIA) kits from Radiosystem Laboratories (Los Angeles, Ca, USA) and were used as recommended by the manufacturer. Crossreactions with related steroids were found to be below 3 percent. Within and between assay coefficient of variation for all assays were below 10 and 20 percent respectively (n = 6). To explore the validity of the method serum samples drawn weekly from each of two oophorectomized animals were analyzed.

Statistics: The Kruskal-Wallis test was used for statistical analysis of the LH-values.

Results

Serum immunoreactive-LH: A serum sample pool from cycle day 1 in the guinea-pig afforded IR-LH levels of 0.86 µg/l. When the analyzed aliquot was doubled the value extrapolated from the standard curve was 1.61, indicating a dose response relation. A rat serum pool (cycle day 1) analyzed at the same time gave an IR-LH value of 2.24 µg/l. In the two oophorectomized animals serum levels of IR-LH after 2 weeks were 0.95–1.28 and 0.94–1.51 µg/l respectively. After 1–2 months the animals showed values of 0.94–1.58 µg/l (mean 1.23 µg/l, n = 10).

Serum immunoreactive FSH: A cycle day 1 pool gave 3.1 µg/l when 50 µl were analyzed and 7.4 µg/l when a 100 µl aliquot was assayed. A similar rat serum pool gave a IR-FSH value of 12 µ/l. One week following oophorectomy the serum IR-FSH concentrations were less than 0.01–0.03 µg/l (n = 2). Samples collected one week to two months after operation showed values between 10.3–21.4 µg/l (mean 14.7, n = 8).

Serum immunoreactive prolactin: Serum samples from 3 animals were analyzed for IR-prolactin during the estrous cycle. All samples showed values below 0.5 µg/l (n = 12), whereas the rat serum pool yielded 20.1 µg/l. Serum prolactin values in three pregnant guinea-pigs yielded results of 0.8–3.0 g/l (mean 2.2, n = 45). No prolactin was found in sera from two males.

Serum estradiol – 17β and progesterone: To validate these assays, serum from the oophorectomized animals were analyzed and none of ten samples gave detectable levels of neither estradiol nor progesterone. The same results were obtained with samples from the two males.

Reproductive hormone levels during the estrous cycle. (Fig. 1 a-d). All animals showed a marked IR-LH surge (at 8–10 p.m.) with values ranging from 0.59 g/l to 2.20 g/l (n = 6) ($p < 0.05$). During other cycle days values of IR-LH ranged from 0.22 to 0.82 µg/l (n = 70, mean 0.44, SD ± 13.5). A characteristic IR-LH pattern is shown in Fig. 1 a. At days IR-LH − 2 to IR-LH 0 high values of IR-FSH were noted (range 22.0–138.7 µg/l, mean 72.7 ± 51.1, n = 17).

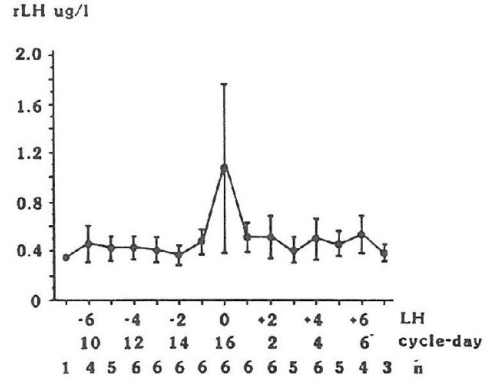


Figure 1 a. Mean IR-LH values from six animals. Note marked IR-LH surge at cycle day sixteen. (Mean, SD and n noted in lowest row).

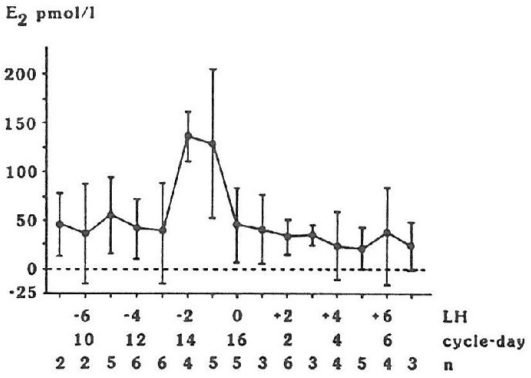


Fig. 1 c. Estrogen fluctuations during the estrous cycle. These are much in parallel with FSH levels, since preovulatory high values are noted. (Mean, SD and n noted in lowest row).

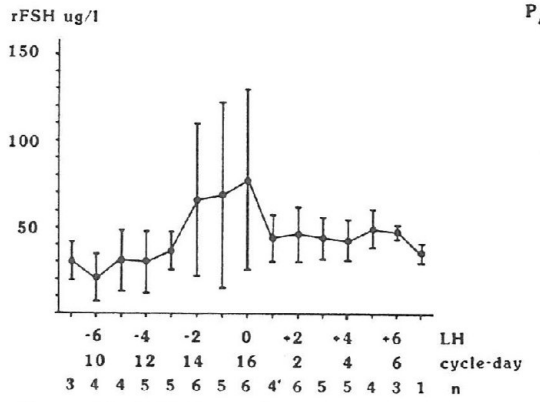
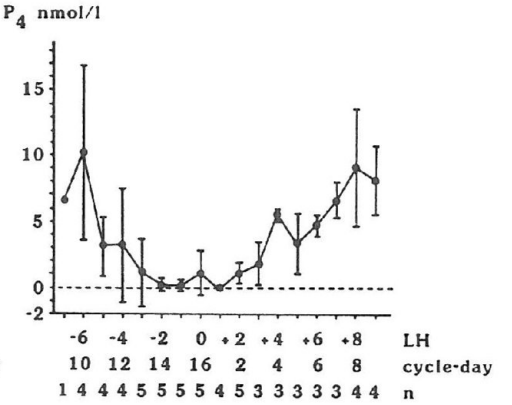


Figure 1 b. FSH fluctuations during the estrous cycle. Mean values from six animals. Note preovulatory peak values. (Mean, SD and n noted in lowest row).



Figur 1 d. Progesterone levels during the oestrus cycle. Note increasing values towards implantation time. (Mean, SD and n noted in lowest row).

Thus a preovulatory peak of IR-FSH was found. The values were about three times higher than those measured during the rest of the estrous cycle (range 0–59.1 µg/l, mean 39.8 ± 16.2, n = 58) (Fig. 1 b).

Estrogen fluctuations were in parallel with IR-FSH changes. Thus preovulatory high values were noted from day IR-LH −2 to IR-LH 0 (range 98.9–205.0 pmol/l, mean 142 ± 33.5, n = 10). There was also a slight postovulatory raise in estrogen values towards the day of implantation (cycle days 5–7). The range of estrogen values at non surge days was 0–116.4 pmol/l (mean 33.9 ± 30.5, n = 54) (Fig. 1c).

Progesterone values gave rise to a biphasic curve with almost undetectable levels around ovulation and increasing serum values towards implantation time. (Peak value 7.4 ± 3.1 nmol/l, n = 14) (Fig. 1 d).

Discussion

The aim of this study was to follow serum levels of reproductive hormones consecu-

tively in individual female guinea-pigs during their estrous cycle. The described method for blood sampling has the great advantage of saving the animals from anaesthesia. It takes, however, some training to perform it. Daily blood samples (0.2–1.0 ml) could be obtained throughout a complete estrous cycle in most animals. Repeated sampling from the same animal offers a more exact determination of hormonal status during the estrous cycle than a single blood sample dated from histological characterization. For the measurement of protein hormones, i e LH-FSH and, prolactin, we employed reagents intended for use in the rat since reference preparations of guinea-pig pituitary hormones are not available. The similarities in immunoreactivity between guinea-pig pituity LH and FSH with corresponding hormones from the rat has previously been explored by *Croix & Franchimont* (1975). Our validations of the assays were only physiological and the levels of IR-LH and IR-FSH we report are not absolute levels but relative ones and the assays have been used to follow changes in the concentrations of these hormones.

IR-LH and IR-FSH increased after oophorectomy. The results have been expressed in rat pituitary hormone equivalents. The lower levels we obtained in guinea-pig serum with these rat reagents probably reflect a crossreactivity with guinea-pig hormones below 100 %. Thus IR-LH was found to be of similar concentration in the two species, whereas IR-FSH levels in the guinea-pig were substantially lower than in the rat. Except for the pregnant animals we were unable to detect IR-prolactin in amounts exceeding 0.5 µg/l. This may indicate that there is a limited crossreaction of guinea-pig prolactin in the rat prolactin assay. There are no previous data on prolactin levels in guinea-pig serum but there is no reason to assume that the guinea-pig should not have circulating prolactin. Furthermore, no estradiol or progesterone responses were obtained after oophorectomy or in male guinea-pig serum indicating absence of matrix effects on these two radioimmunoassays.

The IR-LH pattern we found follows the description given by *Croiz & Franchimont* (1975) showing a preovulatory IR-LH surge. Values are about 1/10 of those reported by these authors, possibly reflecting different methodological setups.

The IR-FSH peak according to our determinations occurs slightly ahead of the IR-LH peak although *Croix & Franchimont* (1975) reported a IR-FSH peak after the IR-LH peak. This difference might be due to the fact that we were able to perform repeated sampling on the same animals thus allowing a more precise determination of the estrous cycle phase. The levels of IR-FSH in µg/l are, however, similar to those of *Croix & Franchimont* (1975).

Most authors have reported undetectable or very low levels of estradiol-17β during the estrous cycle. A preovulatory estrogen peak has been reported (*Croix & Franchimont* 1975, *Garris & Mitchell* 1979). Also in the present study a preovulatory estrogen peak was seen. Values in pmol/l are of about the same magnitude in this study as in the two previously cited reports.

It is well known that progesterone has a biphasic curve with low levels around ovulation (*Croix & Franchimont* 1975, *Garris & Mitchell* 1979, *Hammarström* 1980). The preovulatory progesterone peak described by *Croix & Franchimont* (1975) and *Garris & Mitchell* (1979) could not be demonstrated in the present investigation which is in agreement with the report of *Challis et al.* (1971).

The guinea-pig is thus considered to be a laboratory animal with an estrous cycle which resembles the human female reproductive cycle.The results suggest that the guinea-pig would be the laboratory animal of choice for studies with relevance for human reproduction.

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Summary
A study on hormone levels in daily consecutive serum samples during the guinea-pig estrous cycle was performed. Radioimmunoassay (RIA) of immunoreactive LH, FSH and prolactin (IR-LH, IR-FSH and IR-prolactin) developed for determination of rat pituitary hormone was used as well as RIA systems for estradiol-17β and progesterone. In oophorectomized animals estradiol, progesterone and IR-prolactin were undetectable while mean IR-LH and IR-FSH levels were 1.23 µg/l and 1.47 µg/l respectively as expressed in terms of rat hormone references. Prolactin could not be detected in serum during the reproductive cycle. During estrous cycles progesterone showed a biphasic pattern with undetectable levels (< 0.6 nmol/l) around ovulation. Increased levels were observed at the time of implantation. Estradiol-17β concentrations at non-surge time ranged between 0 and 116 pmol/l and a preovulatory peak (mean peak value 142.2 pmol) was noted. Serum IR-LH levels were between 0.2 and 0.8 µg/l and a preovulatory peak was noted. Furthermore, a preovulatory IR-FSH peak was found (mean peak value 72.7 µg/l). The results indicate a close resemblance to the human reproductive cycle and the guinea-pig is suggested to be the laboratory animal of choice for studies on reproduction.

Sammandrag
I denna studie har dagliga blodprover från marsvin tagits för att följa serumnivåerna av hormoner under reproduktionscykeln. Radioimmunoassay (RIA) av immunoreaktivt LH, FSH och prolaktin (IR-LH, IR-FSH och prolaktin) har mätts med RIA-metoder utvecklade för råtthypofyshormoner, liksom RIA för 17β-östrogen och progesteron. Hos kastrerade marsvin fanns inga mätbara nivåer av östrogen och progesteron i serum, medan medelvärdet av LH och FSH var 1,23 µg/l respective 14,7 µg/l mätt efter råtthormon-referensvärden. Några mätbara nivåer av prolaktin i marsvinsserum kunde ej påvisas. Under reproduktionscykeln visade progesteron ett bifasiskt mönster med låga hormonnivåer vid ovulation (< 0.6 nmol/l) och stigande nivåer noterades vid implantation. 17β-östrogen koncentrationerna i serum varierade mellen 0 och 116 pmol/l med en preovulatorisk peak (medelvärde vid peak 142,2 pmol). IR-LH nivåer i serum varierade mellan 0,2 och 0,8 µg/l och en preovulatorisk peak kunde ses. Vidare uppvisade även IR-FSH en preovulatorisk peak. Resultaten pekar på nära likhet med hormonmönstret hos människa under menscykeln och vi föreslår att marsvin borde vara förstahandsval vid reproduktionsstudier på försöksdjur.

Yhteenveto / K. Pelkonen
Työssä mitattiin marsun kiimakierron kuluessa seerumin hormonipitoisuuksia päivittäin toistuvista verinäytteistä. Menetelmänä käytettiin rotan aivolisäkehormonimäärityksiä varten kehitettyä RIA (radioimmunoassay)-menetelmää LH:lle, FSH:lle ja prolaktiinille (IR-LH, IR-FSH ja IR-prolaktiini) ja rotan aivolisäkkeen estradioli-17β ja progesteronia varten tehtyä RIA-menetelmää. kastroiduilla eläimillä ei voitu havaita estradiolia, progesteronia tai IR-prolaktiinia.IR-LH ja IR-FSH-tasot olivat 1.23 µg/l ja 14.7 µg/l (rotta-hormoniasteikolla). Prolaktiinia ei voitu todeta seerumista lisääntymiskierron aikana. Kiimakierron aikana progesteronia esiinty kaksivaiheisesti. Juuri ovulaation lähellä määrä oli alle mittausherkkyyden (< 0.6 nmol/l). Implantaation aikana taso oli kohonnut. Estradioli-17β-pitoissuudet vaihtelivat 0 ja 116 pmol/l välillä ja siinä havaittiin preovulatorinen piikki (keskimäärin 142.2 pmol). Seerumin IR-LH-tasot vaihtelivat välillä 0.2 ja 0.8 µg/l ja myös siina havaittiin preovulatorinen piikki, kuten myös IR-FSH-ssa (keskimäärin 72.7 µg/l). Tulokset viitaavat huomattavaan samankaltaisuuteen ihmisen ja marsun lisääntymiskierrossa ja puoltavat marsun käyttämistä koeeläimenä lisääntymistutkimukssissa.

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