EFFECT OF HUMAN CHORIONIC GONADOTROPIN ON PREOVULATORY LUTEINIZING HORMONE SURGE AND OVARIAN HORMONE SECRETION IN GILTS\textsuperscript{1,2}

A. Ziecik\textsuperscript{3}, J. E. Tilton, F. Espana\textsuperscript{4} and R. Weigl

North Dakota State University\textsuperscript{5,6}, Fargo 58105

ABSTRACT

The influence of varying doses of human chorionic gonadotropin (hCG) on the preovulatory luteinizing hormone (LH) surge, estradiol-17β (E\textsubscript{2}) and progesterone (P\textsubscript{4}) was studied in synchronized gilts. Altrenogest (AT) was fed (15 mg·head\textsuperscript{-1}·d\textsuperscript{-1}) to 24 cyclic gilts for 14 d. Pregnant mares serum gonadotropin (PMSG; 750 IU) was given im on the last day of AT feeding. The gilts were then assigned to one of four groups (n = 6): saline (I), 500 IU hCG (II), 1,000 IU hCG (III) and 1,500 IU hCG (IV). Human chorionic gonadotropin or saline was injected im 72 h after PMSG. No differences in ovulation rate or time from last feeding of AT to occurrence of estrus were observed. All gilts in Groups I and II expressed a preovulatory LH surge compared with only four of six and three of six in Groups III and IV, respectively. All groups treated with hCG showed a rapid drop (P<.01) in plasma levels of E\textsubscript{2} 11, 17, 23 h after hCG injection when compared with the control group (35 h). The hCG-treated gilts exhibited elevated P\textsubscript{4} concentrations 12 h earlier than the control group (3.1 ± .5, 3.4 ± .72, 3.1 ± .10 ng/ml in groups II, III and IV at 60 h post-hCG vs .9 ± .08 ng/ml in group I; P<.05). These studies demonstrate that injections of ovulatory doses of hCG (500 to 1,500 IU) had three distinct effects on events concomitant with occurrence of estrus in gilts: 1) decreased secretion of E\textsubscript{2} immediately after hCG administration, 2) failure to observe a preovulatory LH surge in some treated animals and 3) earlier production of P\textsubscript{4} by newly developed corpora lutea.

(Key Words: Gilts, Estrogens, Progestogens, hCG, LH.)

Introduction

Pituitary gonadotropic preparations, pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) have been used to induce follicular development or ovulation in pigs. This combined treatment (PMSG + hCG) has been effective following alteration of the estrous cycle with prostaglandins (Polge et al., 1968; Webel et al., 1970; Christenson et al., 1973) or following oral administration of progestogens (Dziuk and Baker, 1962; Dziuk and Polge, 1965).

The pattern of the preovulatory luteinizing hormone (LH) surge after hCG-stimulated ovulation has never been studied. It is believed that LH secretions are regulated by at least two forms of feedback mechanisms; a long loop control mediated by hormone signals from target glands (see Brinkley, 1981 for review), and a short loop or internal feedback mechanism mediated by pituitary hormones themselves (Motta et al., 1969; Docke and Glaser, 1971). Emmanuele et al. (1981, 1985) suggested that LH present in the hypothalamus has a potential role in regulation of pituitary LH release. The intent of the studies reported here was to evaluate the effect of ovulatory doses of hCG (500 to 1,500 IU) on the preovulatory surge of LH and the secretion of estradiol and progesterone following altrenogest and PMSG.

Materials and Methods

Twenty-four cyclic gilts weighing approximately 110 kg were prepared for estrous synchronization by individually feeding 15 mg
active progestin (altrenogest, AT:17-α-allylestratien-4,9,11,17β-ol-3-one) for 14 d. On d 12 of altrenogest treatment, a cannula was inserted into the vena cava via a cephalic vein and exteriorized by passage under the skin to the back to facilitate blood collection. On the last day of altrenogest feeding, all animals were injected with 750 IU PMSG im at 0900 and gilts were assigned randomly to one of four groups: I, control – no hCG (saline); II, 500 IU hCG; III, 1,000 IU hCG; and IV, 1,500 IU hCG. Three milliliters of hCG or saline was injected im 72 h after PMSG injections.

Animals were bled (6 ml) every 12 h from the time of PMSG injection until 1 h before hCG was administered. This hour was established as h 0 of the experiment and referred to the occurrence of maximum estradiol-17β (E₂) in plasma of all groups of gilts after averaging the data. Beginning at that time they were sampled every 2 h for 60 h, then every 12 h until the end of the experiment (h 204). When cannulae lost their patency in three animals 30 to 40 h after hCG injection, these gilts were recannulated. Gilts were checked for estrus with a boar at 2- to 4-h intervals. All animals were mid-ventrally laparotomized 10 d after hCG injections to examine and record ovarian morphology. All fluid-filled follicles larger than 25 mm were classified as cystic.

Heparinized plasma samples were stored at -20 C until their hormone concentration were determined by radioimmunoassay (RIA). Plasma LH was quantified in homologous double-antibody RIA. Antiserum prepared in rabbits against purified porcine LH (Ziecik et al., 1979) was used at an initial dilution of 1:100,000. A highly purified porcine LH preparation (USA-pLH-I-1) was radioiodinated by reaction with 125I-iodine in the presence of chloramine T (Greenwood et al., 1963). Results of the LH-RIA are expressed in terms of purified porcine reference standard USDA-pLH-B1 (biological potency = 1.7 U/mg). There was little competition between pLH and hCG, because hCG (.1 to 100 mIU) failed to inhibit binding of the assay tracer by 50%. Concentrations of 10 and 100 mIU hCG/ tube displaced iodinated LH at a rate of 97 and 73%, respectively. Plasma samples were quantified in three assays with an average intra-assay coefficient of variation of 11.3%. Inter-assay coefficient of variation was 9.7%. Assay sensitivity at 95% binding was .08 ng/ml.

The hCG was measured by record antibody technique with antiserum against the hCG-β subunit. This antiserum did not cross-react with porcine LH (USDA-pLH-B1). Assay sensitivity at 95% binding was 3.3 mIU/ml. Intra- and inter-assay coefficients of variation were 3.6 and 5.7%, respectively.

Concentrations of progesterone were determined in duplicate by RIA, utilizing commercial antiserum. Antibody used for this assay was tested for cross-reactivity with six closely related steroids, all of which exhibited cross-reactivities of <1%. Standard curves of progesterone ranged from 50 to 2,000 pg, with sensitivity of less than 25 pg. Plasma aliquots of 100 μl were extracted with 2 ml of a 2:1 hexane-benzene mixture. Recovery averaged 90.6% with intra-assay and inter-assay coefficients of variation of 4.3 and 10.6%, respectively.

Concentrations of estradiol-17β were determined in duplicated aliquots of plasma (250 μl) by radioimmunoassay. Cross-reactivity of the antibody with estrone, estriol and estradiol-17α was 1.7, 1.2 and .8%, respectively. Testosterone and progesterone cross-reacted against the antibody <.10%. Standard curves of estradiol-17β ranged from 2.5 to 200 pg with a sensitivity of <2.5 pg. Plasma samples were extracted with 4 ml of benzene. Recovery of labeled estradiol-17β from control plasma averaged 80.5 ± 1.7% based upon results from eight assays. Intra- and inter-assay coefficients of variation were 6.9 and 15.2%, respectively, based upon the analysis of a selected sample which was run with each assay.

Statistical Analysis. Plasma concentrations were related to onset of the LH preovulatory surge as estimated by the LH surge initiating rise (LH-SIR) parameter proposed by Testart et al. (1982). In our studies, LH-SIR corresponded to the time when the plasma LH amplitude was twice the mean of the four preceding values. Preovulatory LH surge peak was estimated to occur when the plasma concentration reached at least a fivefold value calculated for SIR and duration of the LH release was longer than 10 h.

---

7 New England Nuclear, Cambridge, MA.
8 Micromedic Systems Inc., Horsham, PA.
9 Arnel Products Co., New York, NY.
10 Sigma Chemical Co., St. Louis, MO.
11 Eli Lilly Co., Indianapolis, IN.
Data are presented as means ± SE. Plasma hormone data were analyzed as a split-plot (Gill and Hafs, 1971) design with repeated measurements over time using the General Linear Model (GLM) procedure of SAS (1982). The classification variables for the whole and split-plot portions of the analysis for each plasma hormone were the dose of hCG and hours of collection, respectively. Duncan's new multiple range test was used to compare means among doses of hCG for each hour of blood collection at the .05 level of probability.

Results

The time to onset of estrus ranged from 94.7 h for Group I to 101.5 h for Group IV (table 1). One gilt in Group III and two gilts in Group IV failed to exhibit estrus. Corpora lutea were found in all animals used in the experiment, including gilts without a preovulatory LH surge, and did not vary significantly in numbers among groups. All groups contained at least one gilt with follicular cysts, but Groups III and IV each had two gilts with recognizable cysts. The plasma concentrations of hCG in animals injected with 1,000 and 1,500 IU hCG were generally greater than in the 500 IU hCG group. Also duration of the presence of detectable amounts of hCG in plasma was greater for the higher doses (figure 1). Maintenance of detectable hCG in blood varied from 33.6 ± 1.5 (Group II) to 44.6 ± 1.9 h (P<.05) in Group IV. Plasma hCG increased immediately after administration and reached maximum concentrations of 28 ± 14, 24 ± 13 and 13 ± 2 mg/ml after 13, 15 and 21 h in Groups IV, III and II, respectively. A characteristic second peak of hCG in plasma occurred in all hCG-treated groups 31 to 35 h after injection (figure 1). The preovulatory LH surge did not occur in two gilts in Group III and in three gilts in Group IV (figure 2). The incidence and duration of preovulatory LH surge, intervals from injection of hCG and from peak concentrations of E2 to LH-SIR and maximum levels of LH and E2 are shown in table 2. Duration of time from hCG or saline treatment to LH-SIR varied from 24 ± 6 (Group I) to 13 ± 5 h (Group III), but these differences were not statistically significant. In one gilt in Group II a “small” LH surge was detected (1.04 ng/ml). In five gilts without a preovulatory LH surge only one exhibited attenuated E2 (peak value 15 pg/ml, gilt 65–1, figure 2) compared with means of Groups I, II, III and IV (table 2). This gilt was not detected in estrus. Plasma estrogens increased 48 to 72 h before the preovulatory LH range in all remaining animals (figure 3). In all groups treated with hCG, plasma concentrations of E2 rapidly dropped (P<.01), 11, 17, 23 h after hCG injection when compared with the control group (figure 4). In 19 animals having a preovulatory surge of LH, the LH-SIR never occurred before peak E2.

Plasma progesterone was less than 2 ng/ml in all groups (figure 4) until 59 h after the hCG injection (Groups II, III, IV, 3.1 ± .05, 3.5 ± .72, 3.1 ± .10 ng/ml, respectively vs .9 ± .08 ng/ml in Group I, P<.05). Progesterone started to rise in the control gilts 12 h later, reaching a concentration of 3.2 ± .81 ng/ml by 72 h. Concentration of progesterone in Group IV (1,500 IU hCG) was higher than in Groups I, II and III at 96 h (P<.05), throughout h 108, 120, 132 (P<.01), 144 and 156 (P<.001) and 168 (P<.05).

| TABLE 1. EFFECT OF ALTRENOGEST (AT) AND GONADOTROPHINS ON REPRODUCTIVE ACTIVITY |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Item                           | I (Control)     | II (500 IU hCG) | III (1,000 IU hCG) | IV (1,500 IU hCG) |
| Proportion of gilts in estrus  | 6/6             | 6/6             | 5/6             | 4/6             |
| Time from last AT feeding h (range) | 94.7 ± 4.0^a  | 101.2 ± 2.7    | 98.0 ± 2.6      | 101.5 ± 2.9     |
|                               | (77–107)        | (94–107)        | (94–107)        | (96–107)        |
| No. of corpora lutea (range)   | 15.3 ± 2.1^a    | 15.3 ± 1.8     | 16.3 ± 2.3      | 12.5 ± 0.7      |
|                               | (6–22)          | (12–24)         | (8–23)          | (9–14)          |

^a Values are means ± SE.
Discussion

The PMSG-stimulated follicular growth is accompanied by an increased production and release of ovarian steroids. Dial et al. (1984) showed a positive correlation between dose of PMSG and peak blood levels of E2 and between dose and number of induced ovulations in gilts. In our study, all groups of gilts were injected with the same dose of PMSG (750 IU) and concentrations of E2 prior to hCG injection did not differ significantly (table 2). Also, no differences in ovulation rate were observed (table 1). Although hCG has been widely used for induction of ovulation in pigs, the effect of different doses of this gonadotropin on the preovulatory LH surge and secretion of estradiol and progesterone have never been studied. Injected hCG was observed in the blood for 33 to 44 h, depending on dose. We also found a coincidental occurrence of a second peak of hCG in the peripheral circulation of the treated animals after averaging the data. Human chorionic gonadotropin was shown to fluctuate greatly with animals, and the two-peak picture (Group III and IV) could possibly be the consequence of averaging rather than a representation of what is occurring in the individual pig. However, two-peak patterns of hCG concentrations after im injection were observed in individual pigs in our preliminary study on validation of RIA methods for determination of hCG in pig serum (A. J. Ziecik and J. E. Tilton, unpublished information). This aspect of our study is worthy of discussion. In our opinion, the delayed appearance of high concentrations of hCG in blood cannot be explained by simply an additional release from an injected hormone, although such a possibility should not be ruled out. The study of hCG metabolism and rate of clearance in humans (Yen et al. 1968; Rizkallah et al., 1969), monkeys (Rao, 1985) and rats (Markkanen et al., 1979) has demonstrated that the rate of disappearance of hCG from serum follows a biphasic pattern. The half-life of hCG varies, depending upon the physiological status of the animal under investigation. For example, it is much shorter in non-pregnant than pregnant monkeys (Rao, 1985). Injected hCG can be distributed between the plasma, kidney and various target tissues. Recently Moyle et al. (1986) found that hCG is absorbed or bound to cell membranes lacking hCG/LH receptors in human and rat tissues. It is likely that some hCG is bound to the target tissue several hours...
Figure 2. Concentration of LH, hCG, estradiol-17β (E₂) and progesterone (P₄) in two gilts having no preovulatory LH surge. Broken vertical line shows time of injection (hours + 1).

Figure 3. Patterns of LH, hCG, estradiol-17β (E₂) and progesterone (P₄) level exhibited by a representative gilt (53-2), which showed preovulatory LH surge after injection of 1,000 IU hCG. Broken vertical line indicates time of injection.
Evidence that dissociation but not intracellular degradation is the major pathway for removal of receptor-bound hCG in luteal cells was shown in rats (Rajan and Menon, 1985). Furthermore, Ziecik et al. (1986) reported that the myometrium is a target tissue for LH and hCG in the pig, in addition to the ovaries. Myometrial cell membrane receptors bind both gonadotropins with the same affinity ($K_a \times 10^{10} \text{ M}^{-1}$) and specificity as luteal receptors. These data provide additional support for the suggestion that hCG/LH has other functions besides formation of the corpus luteum, and may explain the occurrence of additional peak(s) of hCG in the systemic circulation of the treated animals.

Injections of ovulatory doses (500 to 1,500 IU) of hCG had three distinct effects on events concomitant with the occurrence of estrus in gilts: 1) decrease secretion of estradiol-17β immediately after hCG injection, 2) failure to observe a preovulatory LH surge in some treated animals and 3) earlier production of progesterone by newly developed corpora lutea.

Data from in vitro experiments suggest that steroid biosynthesis in the porcine follicle during the estrous cycle proceeds to estrogens and later stops at progesterone (Meinecke et al., 1984). Those authors concluded that the Δ^5-steroid biosynthetic pathway is relatively unimportant in ovarian steroid formation in the pig. The steady decrease of estradiol-17β from 11 h after hCG injections reflects the inhibition of steroidogenesis in preovulatory follicles. These data confirm in vitro results of Ainsworth et al. (1980), which indicated that hCG terminates estradiol synthesis by preovulatory follicles in pigs in a manner similar to the endogenous LH surge in cows (Staigmiller et al., 1982) and sheep (Moor, 1974), or after exposure to LH in vivo or in vitro in rats (Lieberman et al., 1975; Hillensjo et al., 1976; Katz and Armstrong, 1976; Hamberger et al., 1978; Goff and Henderson, 1979) and hamsters (Saidapur and Greenwald, 1978). It is hypothesized that gonadotropins (LH or hCG) inhibit estrogen production by causing depletion of the aromatizable substrate (Dieleman et al., 1983a,b) and(or) inhibit transfer of thecal androgens to granulosa cells (Evans et al., 1981) as well as decrease the aromatizing capacity of the membrane granulosa (Fortune and Hansel, 1979).

In pigs, data are equivocal concerning whether the preovulatory increase in LH
secretion begins before circulating estrogens reach a maximum (Henricks et al. 1972) or begin to decrease (Brinkley, 1981). Van de Wiel et al. (1981) suggested that the preovulatory LH surge begins about 4 h before estradiol-17β reaches its highest concentration in the blood. In our study, SIR of LH never occurred before the peak concentration of estradiol-17β in the blood was reached, which is consistent with Brinkley (1981), who reported that estradiol-17β secretion reached maximum 1 d before the onset of estrus and the preovulatory LH surge. We suggest that pigs, like dogs (Concannon et al., 1979) and rats (Aiyer et al., 1974; Hender-
son et al., 1977) are species in which decreasing concentrations of estradiol in blood trigger initiation of the preovulatory LH surge. In our study, the preovulatory LH surge did not occur in five hCG-treated gilts. One explanation is that sufficient time had not elapsed from the estrogen trigger to enable endogenous LH to be produced. Among those five gilts, only one had low concentrations of E₂, so the threshold values required for elicitation of a LH surge could not be reached. The remaining four (one received 1,000 IU, three others received 1,500 IU hCG) had a typical preovulatory rise of E₂.

For a second explanation, we suggest that injected hCG blocked the preovulatory LH surge in these gilts. Evidence suggesting an inhibitory effect of circulating LH on its own synthesis and release by the pituitary has been drawn from studies where LH has been placed stereotactically in the hypothalamus, or in the medium eminence, or where hCG or LH has been administered systemically. After such manipulations, significant decreases in circulating LH and pituitary LH content have been noted (Hirono, 1972; Ying and Meyer, 1972; Miyake et al., 1978). In women (Miyake et al., 1977) and gilts (Guthrie and Bolt, 1983), given hCG during the luteal phase, immunoreactive LH falls significantly.

The appearance of relatively high concentrations of progesterone (> 3 ng/ml) in hCG-treated gilts 12 h earlier than in the control group apparently indicates an earlier completion of the process of luteinization of granulosa cells after an hCG injection (figure 4). An effect of the hCG treatment on later progesterone secretion by newly developed corpora lutea was not found; however, gilts injected with a high dose of hCG had higher progesterone concentrations in blood plasma. Our results support the early hypothesis of Nalbandov (1970) and Hansel et al. (1973) of the importance of a first signal luteinization and corpora lutea function, which are essentially independent during early luteal phase of the estrous cycle in pigs (du Mesnil du Buisson and Leglise, 1963; Spies et al., 1967). Early detection of elevated progesterone concentrations in hCG-treated gilts when compared with untreated controls suggests that hCG caused ovulation in those animals independent of the hypothalamus-hypophyseal unit.

In summary, these results indicate that hCG has definitive effects on E₂ and P₄ secretion in pre- and post-ovulation ovaries in pigs. We also observed the absence of preovulatory LH surge in some hCG-treated gilts. To explain these findings we proposed two possible hypothesis, a short loop regulation, and (or) a disturbance of the estradiol stimulation of the hypothalamus-hypophyseal axis. Final explanation of these phenomena requires further research.

Literature Cited


Alyer, M. S., G. Fink and F. Greig. 1974. Changes in the sensitivity of the pituitary gland to luteinizing hormone releasing factor during the estrous cycle of the rat. J. Endocrinol. 60:47.


Endocrinology 104:1540.


Van de Wiel, D. F., M. J. Erkens, W. Koops, E. Wos


