Potential role of activin A in follicular development during the second half of pregnancy in the golden hamster: utero-placental source of activin A

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Abstract

Numerous antral follicles develop during the second half of pregnancy in the golden hamster. However, mechanisms regulating follicular development during this period are unknown. Because inhibin and activin are related to follicular development, these hormones were studied to gain insight into any potential roles in follicular development. Plasma inhibin A and B suddenly increased from day 8 of pregnancy, reached peak levels on day 10 and gradually declined to term. Plasma activin A gradually increased from day 8 to day 15 of pregnancy, and this was followed by an abrupt decrease at day one of lactation. Ovariectomy on day 12 of pregnancy rapidly reduced plasma inhibin A and B, but not activin A levels. Hysterectomy or placentectomy on day 12 of pregnancy caused an abrupt decrease in the levels of plasma activin A and FSH, but not inhibin A and B at 6 h after surgery. Hysterectomy also induced atresia of large antral follicles at 24 h after surgery. These results indicate that antral follicles are the main source of circulating inhibin A and B, whereas uteri and placentae are the main source of circulating activin A. These results suggest that increased levels of activin A may be involved in folliculogenesis in the ovary during the second half of pregnancy in the golden hamster.

Journal of Endocrinology (2002) **172,** 247–253

Introduction

Inhibins and activins are structurally related dimeric proteins first identified in ovarian follicular fluid (Robertson *et al.* 1985, Vale *et al.* 1986). Inhibins are heterodimers consisting of a common α subunit and either a βA or βB subunit, resulting in inhibin A or inhibin B respectively (De Jong 1988). Inhibins are important factors in regulating secretion of follicle stimulating hormone (FSH) in mammals. Inhibins, through the regulation of FSH secretion, have been reported to be key factors for determining species-specific ovulation rates (Taya *et al.* 1999, Taya 1993, Kishi *et al.* 1996, Taya *et al.* 1996). Activins are dimers of the β subunits, with three forms currently identified: activin A (βA-βA), activin B (βB-βB) and activin AB (βA-βB) (Ying 1988). Activins are important proteins that have several physiological functions in reproduction, such as stimulation of FSH secretion (Vale 1986, Schwall *et al.* 1989) and increasing the number of FSH receptors (Hasegawa *et al.* 1988, Nakamura *et al.* 1993). Activin is also well known as an important factor that may act in the early development in the formation of the mesoderm (Albano *et al.* 1990, Smith *et al.* 1990).

During the second half of gestation in the golden hamster, numerous antral follicles develop (Greenwald 1964), but all large antral follicles degenerate at the end of pregnancy. Post partum ovulation, as seen in rats (Ying *et al.* 1973), does not occur in the golden hamster (Greenwald 1965) and large antral follicles do not reappear until the end of lactation. The proliferation of numerous antral follicles during the second half of pregnancy in the golden hamster is also markedly different compared with the pattern in the pregnant rat (Greenwald 1966, Taya & Sasamoto 1977). Mechanisms of follicular dynamics during the second half of pregnancy in the golden hamster are unclear.

In the present study, to investigate mechanisms of follicular dynamics in the second half of pregnancy in the golden hamster, secretory patterns of inhibin A, inhibin B and activin A, and the source of these hormones were determined.

Materials and Methods

Animals

Adult female golden hamsters (*Mesocricetus auratus*), maintained on a 14 h light:10 h darkness schedule (lights on from 0500 h to 1900 h) were mated after two consecutive 4-day oestrous cycles. Female hamsters were placed with males on the evening of pro-oestrus, and the presence of sperm in the vagina was designated as day 0 of pregnancy. In our colony, gestation lasts 15 days, with most hamsters delivering on day 15 of pregnancy. The day of parturition was designated as day 0 of lactation. Food and water were available *ab libitum*.

Experimental design

Plasma concentrations of inhibin A, inhibin B and activin A during the second half of pregnancy Groups of animals ($n=5$) were decapitated daily at 1100 h from day 8 of pregnancy to day 1 of lactation, and trunk blood and ovaries were collected. Blood samples were centrifuged immediately at 1700 g for 15 min at 4 °C and plasma was separated and stored at −20 °C until assayed. Ovaries, uteri and placentae were removed immediately after death and weighed. Ovaries, uteri and placentae were stored at −20 °C in 1 ml 0·85% (w/v) NaCl solution. Ovaries, uteri and placentae were thawed on ice, homogenized (UR 200P; Tomy, Tokyo, Japan) and centrifuged at 20 000 g for 30 min at 4 °C. Supernatants were stored at −20 °C until assayed for activin A.

Ovarian follicular development during pregnancy To determine the number of large healthy antral follicles in the ovary during the second half of pregnancy, 10 IU human chorionic gonadotrophin (hCG) dissolved in 0·2 ml 0·85% (w/v) NaCl solution were injected into the jugular vein under ether anaesthesia (2200 iu/mg; Sankyo Zoki Co., Tokyo, Japan) on days 2, 4, 6, 8, 10, 12 or 14 of pregnancy. Animals were decapitated at 18–20 h after hCG injection, and the oviducts were examined for oocytes.

Plasma concentrations of inhibin A, inhibin B and activin A after surgery To examine the sources of inhibin A, inhibin B and activin A, pregnant hamsters underwent one of the following: ovariectomy, placentectomy (placentae with decidual tissues and fetuses were removed, and the uterus was not sutured), hyster-

ectomy (uterus, placentae with decidual tissues and fetuses were removed), ovariohysterectomy or sham-operation under ether anaesthesia at 1100 h on day 12 of pregnancy. Just before, and 1, 3 and 6 h after surgery, groups of animals were decapitated and trunk blood was collected. Blood samples were centrifuged immediately at 1700 g for 15 min at 4 °C, and plasma was separated and stored at −20 °C until assayed.

The number of large healthy antral follicles after hysterectomy on day 12 of pregnancy To examine the effect of hysterectomy on follicular activity, pregnant hamsters were hysterectomized at 1100 h on day 12 of pregnancy, then 10 IU hCG dissolved in 0·2 ml 0·85% (w/v) NaCl were injected into the jugular vein under ether anaesthesia at 1100 h on day 13 of pregnancy (24 h after the surgery). Animals were decapitated 18–20 h after hCG injection, and the oviducts were examined for oocytes.

Enzyme linked immunoabsorbent assay (ELISA) of inhibin A, inhibin B and activin A Concentrations of inhibin A, inhibin B and activin A were measured by ELISA (Serotec, Oxford, Oxon, UK) as described previously (Knight *et al.* 1996, Ohshima *et al.* 1999). Amounts of inhibin A and inhibin B were expressed in terms of recombinant human inhibin A and inhibin B respectively. Amounts of activin A were expressed in terms of recombinant activin A.

RIA of luteinising hormone (LH), FSH, progesterone, and oestradiol-17β Concentrations of LH and FSH in plasma were measured using NIDDK RIA kits for rat LH and FSH as described previously (Bast & Greenwald 1974*a*). Iodinated preparations were rat LH-I-5 and FSH-I-5. The antisera used were anti-rat LH-S-9 and anti-rat FSH-S-11. The intra- and interassay coefficients of variation were 8·9% and 6·7% for LH and 4·4% and 14·6% for FSH respectively.

Concentrations of progesterone and oestradiol-17β in plasma were measured by double antibody RIA systems using ^{125}I-labelled radioligands as described previously (Taya *et al.* 1985). Antisera against progesterone (GDN 337; Gibori *et al.* 1977) and oestradiol-17β (GDN 244; Korenman *et al.* 1974) were kindly provided by Dr G D Niswender (Colorado State University, Fort Collins, CO, USA). The intra- and interassay coefficients of variation were 6·3 and 15·4% for progesterone and 3·7% and 6·2% for oestradiol-17β respectively.

Statistics

All data were expressed as means ± S.E.M. One-way ANOVA was performed, and the significance between two means was determined by Student's *t*-test or Cochran-Cox test, and the significance among more than
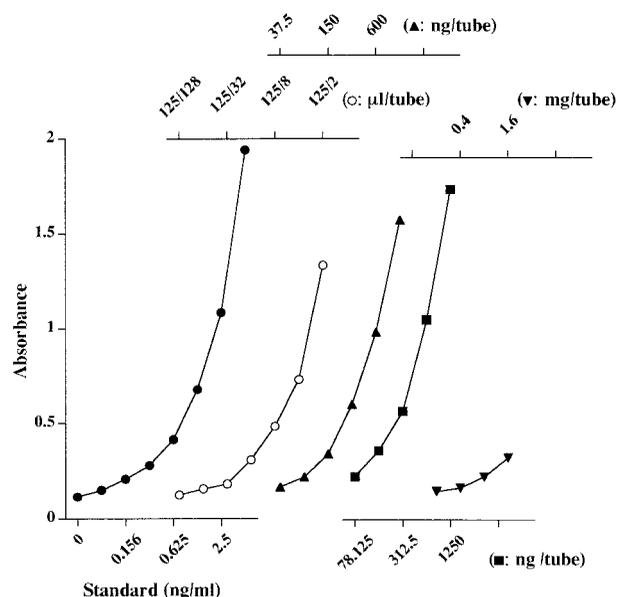
Figure 1 ELISAs for activin A. Dose–response curves of activin A in standard (●), plasma (○), placental homogenates (▲), uteri homogenates (■) and ovarian homogenates (▼) on day 12 of pregnancy in the female golden hamsters.

two means was determined by Duncan's Multiple Range test (Steel & Torrie 1960). A value of $P<0.05$ was considered statistically significant.

Results

Characterization of activin A ELISA (Fig. 1)

Competition between labelled and unlabelled antigen with the ovarian homogenates, peripheral plasma, placental homogenates and uterine homogenates of pregnant hamsters produced excellent dose–response curves in activin A assays. Each curve was reliably parallel with the respective standard curve, indicating that it was possible to measure the concentration of activin A of the female golden hamster using this kit. These results also clearly demonstrated that placenta and uterus of pregnant golden hamsters contained large amounts of activin A.

Induction of ovulation during the second half of pregnancy (Table 1)

Although a constant number of follicles ovulating in response to hCG (16.0 ± 0.7 on day 2; 15.8 ± 1.5 on day 8) was maintained in the ovary from day 2 to day 8 of pregnancy, the number of follicles ovulating in response to hCG approximately doubled on day 10 of pregnancy (33.2 ± 3.2) and was maintained until day 14 of pregnancy.

Table 1 Induction of ovulation by 10 IU human chorionic gonadotrophin (hCG) administered on various days during pregnancy in the golden hamster. All hamsters (5 per group) ovulated at the expected time. Results are means ± S.E.M.

Day of pregnancy	No. of oocytes/ ovulating hamster
2	16.0 ± 0.7[a]
4	15.4 ± 2.2[a]
6	18.4 ± 1.4[a]
8	15.8 ± 1.5[a]
10	33.2 ± 3.2[b]
12	34.6 ± 3.8[b]
14	27.4 ± 4.4[b]

Values with different superscripts are significantly different ($P<0.05$, Duncan's test).

Plasma concentrations of inhibin A, inhibin B, activin A, FSH, LH, oestradiol-17β and progesterone during the second half of pregnancy (Fig. 2)

Plasma concentrations of inhibin A and inhibin B abruptly increased from day 8 and reached maximum levels on day 10 of pregnancy. Thereafter, plasma concentrations of inhibin A and inhibin B gradually decreased until day 1 of lactation.

Plasma concentrations of activin A significantly increased from day 8 (0.55 ± 0.03 ng/ml) to day 9 (1.48 ± 0.31 ng/ml) of pregnancy. Thereafter, plasma concentrations of activin A increased gradually, and reached the maximum level on day 15 of pregnancy; this was followed by an abrupt decrease with delivery.

Plasma concentrations of FSH and LH remained at basal levels and the levels were unchanged during the second half of pregnancy.

Plasma concentrations of oestradiol-17β increased from day 8 and reached plateau levels on day 9 of pregnancy. The plateau level was maintained until day 13, and was followed by an abrupt decline to below the limit of detection on day 15 of pregnancy.

Plasma concentrations of progesterone were maintained at plateau levels from day 8 to day 10 of pregnancy. Thereafter, plasma concentrations of progesterone further increased and reached the maximum level on day 13, followed by an abrupt decline on day 15 of pregnancy.

Plasma concentrations of inhibin A, inhibin B, activin A and FSH after ovariectomy, ovariohysterectomy, placentectomy or hysterectomy (Fig. 3)

Plasma concentrations of inhibin A and inhibin B declined markedly after ovariectomy or ovariohysterectomy,
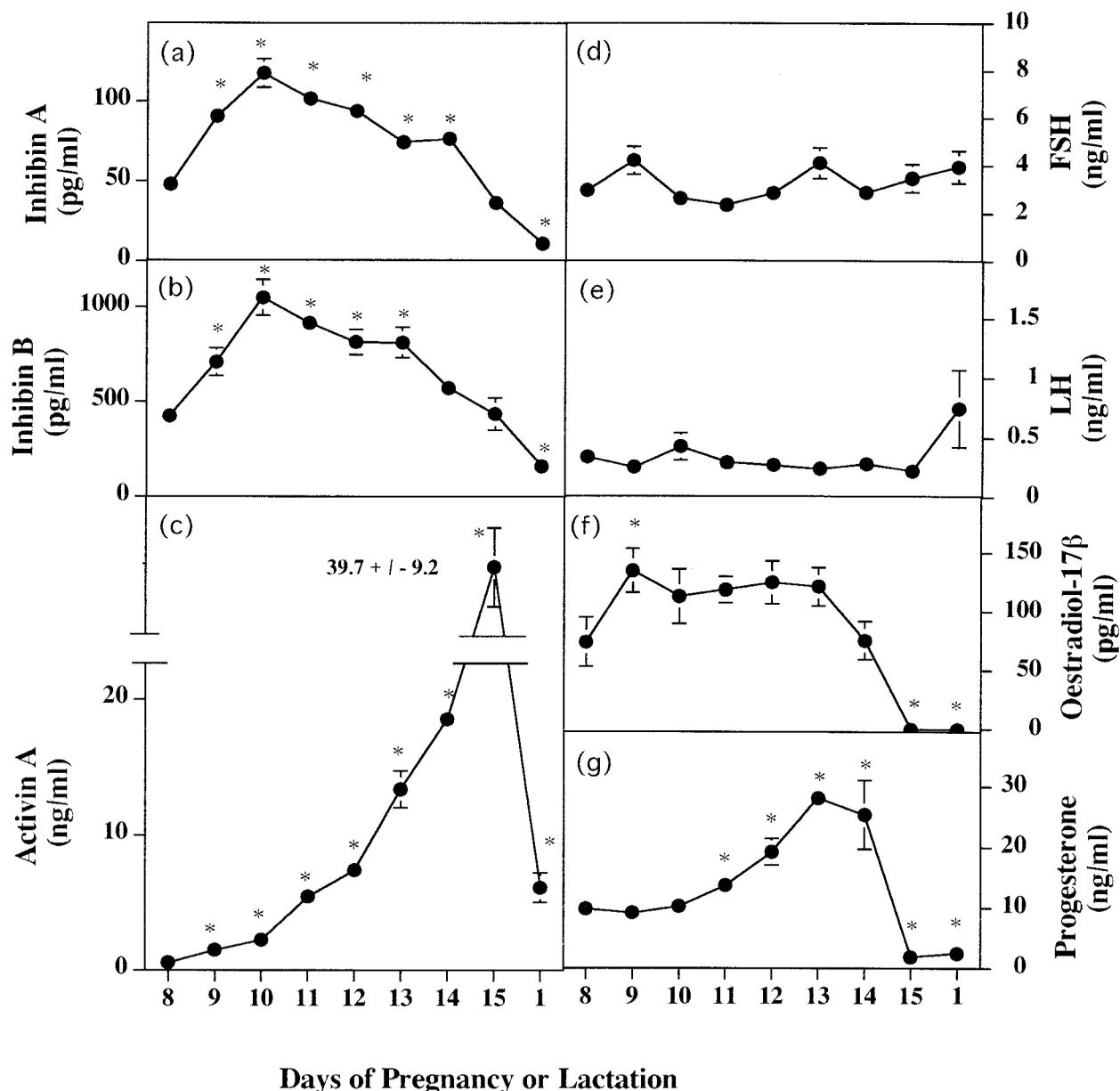
Figure 2 Changes in plasma concentrations of (a) inhibin A, (b) inhibin B, (c) activin A, (d) FSH, (e) LH, (f) oestradiol-17β and (g) progesterone during the second half of pregnancy and in early lactation in the golden hamster. Each value represents the mean ± S.E.M. of five animals. *$P<0.05$ compared with the value at day 8 of pregnancy (Duncan's Multiple Range test for inhibin A, inhibin B, FSH, LH and oestradiol-17β; Student's *t*-test for activin A and progesterone).

whereas there were no differences between sham-operation and placentectomy or hysterectomy.

Plasma concentrations of activin A in the placentect-omized, hysterectomized and ovariohysterectomized groups significantly declined at 1 h after operation and the level gradually decreased to 34·6, 8·6 or 9·6% of sham-operated controls, respectively, at 6 h post surgery. On the other

hand, plasma concentrations of activin A in the sham-operated and ovariectomized groups did not show any significant changes throughout the 6 h following surgery.

Plasma concentrations of FSH increased markedly at 6 h after ovariectomy and ovariohysterectomy, whereas plasma concentrations of FSH decreased at 6 h after placentectomy or hysterectomy.
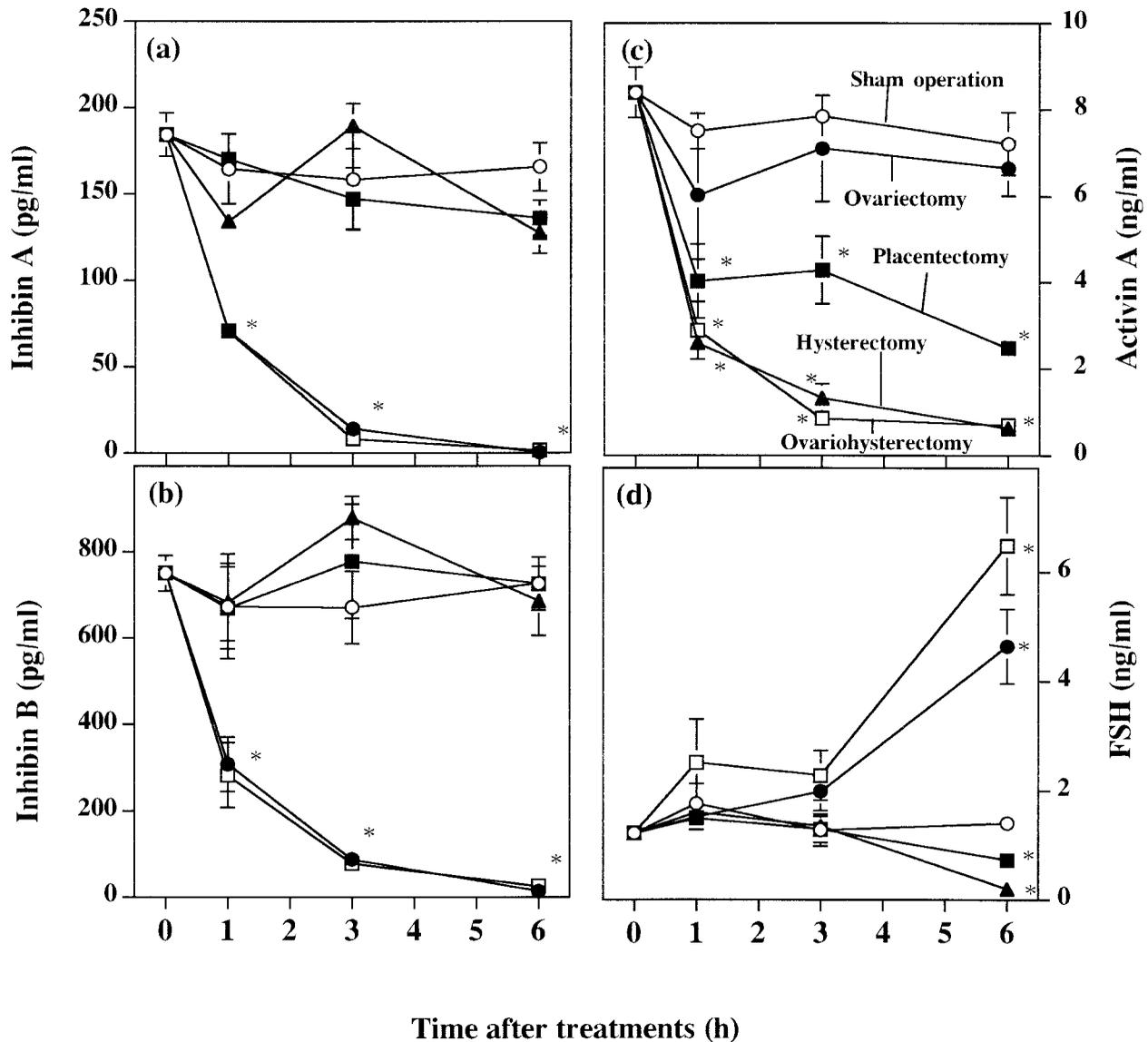
Figure 3 Plasma concentrations of (a) inhibin A, (b) inhibin B, (c) activin A and (d) FSH after ovariectomy (●), placentectomy (■), hysterectomy (▲), ovariohysterectomy (□) or sham operation (○) at 1100 h on day 12 of pregnancy in the golden hamster. Each value represents the mean ± s.e.m. of five observations. *P<0·05, significantly different from time-matched sham-operated group (Student's *t*-test for inhibin A and activin A; Duncan's New Multiple Range test for inhibin B and FSH).

Induction of ovulation after hysterectomy (Table 2)

Hysterectomy at 1100 h on day 12 of pregnancy signifi-cantly reduced the number of follicles ovulating in response to hCG as compared with the sham–operated group.

Discussion

This study clearly demonstrates that plasma concentrations of inhibin A, inhibin B, oestradiol-17β and activin A

Table 2 Induction of ovulation in sham-operated or hysterectomized hamsters by 10 IU hCG on day 13 of pregnancy. Pregnant hamsters were hysterectomized or sham-operated on day 12 of pregnancy

Treatment group	Number of ova on day 14	Hamsters ovulating/ hamsters examined
Sham-operated	25·4 ± 1·4	5/5
Hysterectomized	1·5 (1, 2)	2/6

increased during the second half of pregnancy in the golden hamster. These changes in circulating inhibins correlated well with the number of healthy large antral follicles ovulating in response to hCG. Greenwald (1967) demonstrated that the number of follicles larger than 415 µm in diameter had a relationship with the number of ovulations induced by hCG throughout pregnancy in the hamster. Previous studies (Kishi *et al*. 1995, Ohshima *et al*. 1999) also demonstrated that the changing pattern of plasma concentrations of inhibin corresponded well with the number of healthy large antral follicles during the oestrous cycle. Similar correlations of inhibin and follicular development have also been observed in female rats in the various reproductive stages (Taya *et al*. 1989). In the present study, after ovariectomy but not after hysterectomy or placentectomy, plasma concentrations of inhibin A and inhibin B decreased abruptly. These observations clearly indicate that the ovarian antral follicles are responsible for the increase in plasma inhibins during the second half of pregnancy in the golden hamster. On the other hand, after placentectomy or hysterectomy, but not after ovariectomy, plasma concentrations of activin A markedly decreased 1 h after surgery, and placental and uterine homogenates contained a large amount of activin A. These results indicate that activin A is likely to be secreted from placentae and uteri during the second half of pregnancy in the golden hamster. Results in a previous paper demonstrated that activin βA mRNA was expressed in the rat placenta (Gu *et al*. 1995). Previous reports demonstrated that the treatment of granulosa cells with activin increased FSH receptor (Hasegawa *et al*. 1988, Nakamura *et al*. 1993). In addition, hysterectomy on day 12 of pregnancy, which lowered activin A secretion within 1 h, noticeably reduced the number of ova shed in response to hCG. The present study, in conjunction with those of Hasegawa *et al*. (1988) and Nakamura *et al*. (1993), suggests that elevated activin A concentrations secreted from uteri and placentae increase ovarian FSH receptors resulting in follicular recruitment in the presence of the low but unchanging levels of FSH during the second half of pregnancy in the golden hamster. In the present study, however, hysterectomy also decreased FSH secretion. Therefore, lowered FSH secretion may also be responsible for the decrease in ovulation rate after surgery.

It has been demonstrated that atresia destroys all large multilayered and vesicular follicles on the day after parturition (Greenwald 1964). Changes in plasma concentrations of oestradiol-17β in the present study suggest that all large antral follicles have already become atretic on day 15 of pregnancy although plasma concentrations of activin A are still high. The cause of atresia at the end of pregnancy is still unknown. A number of studies have indicated that activin supports the development of follicles. However, a previous study has demonstrated that an injection of a high dose of activin A into the ovarian intrabursal spaces causes follicular atresia (Woodruff *et al*.

1990). Therefore, activin at high concentrations may induce follicular atresia. Because a very high level of plasma activin coincided with the decline of plasma oestradiol on day 15 of pregnancy, the elevated activin A may be a cause for follicular atresia before parturition.

In the present study, a marked increase in the number of large antral follicles was found during the second half of pregnancy in the golden hamster, although plasma concentrations of FSH and LH were unchanged during this time. The levels of circulating FSH and LH during the second half of pregnancy agree with a previous report (Bast & Greenwald 1974*b*). The present study also indicated that plasma concentrations of FSH decreased at 6 h after placentectomy and hysterectomy probably due to reduced circulating levels of activin A. Doi *et al*. (1992) demonstrated that exogenous activin A induced an increase in the secretion of FSH from the pituitary gland in the immature rat. Schwall *et al*. (1989) have also reported that daily subcutaneous injections of recombinant human activin A for 3 days to immature female rats caused a marked increased in serum FSH levels. These results suggest that activin A stimulates FSH secretion and maintains the basal levels of FSH in spite of high levels of inhibin A and inhibin B in the second half of pregnancy in the golden hamster.

In conclusion, the present study clearly demonstrates that during the second half of pregnancy in the golden hamster, a large amount of inhibin A and inhibin B are secreted mainly from the ovarian follicles, whereas activin A is secreted mainly from placentae and uteri. Furthermore, a large amount of activin A may be an important factor in the stimulation of follicular development during the second half of pregnancy in the golden hamster.

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