*Acta Veterinaria Hungarica 49 (4), pp. 443–450 (2001)*

# OVARIAN STIMULATION AND ULTRASOUND-GUIDED OOCYTE RETRIEVAL IN BABOON (*PAPIO CYNOCEPHALUS ANUBIS)* DURING PITUITARY SUPPRESSION WITH A GnRH AGONIST*

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(Received April 18, 2001; accepted September 5, 2001)

The objective of this study was to investigate whether baboon females respond to an ovarian stimulation protocol incorporating pituitary suppression with a GnRH agonist (GnRHa) and either highly purified human FSH (hphFSH) or recombinant human FSH (rhFSH) with follicular development and oocyte maturation. A modified human ovulation induction protocol was applied to 5 adult female baboons with a history of regular menstrual cycles (33–34 days). A long-acting GnRHa implant containing goserelin acetate was placed subcutaneously (s.c.) on Days 22–24 of their menstrual cycle. Concentrations of serum oestradiol (E2), progesterone (P4) and human FSH were obtained by ELISA. Menses occurred ~ 10 days after GnRHa implantation. Daily hphFSH or rhFSH (75 IU i.m.) treatments were started ~ 10 days following menses. When the majority of follicles were ≥ 5 mm in diameter and the E2 levels had reached a maximum, hCG (2000 IU i.m.) was administered to induce final maturation of oocytes and ovulation. Thirty to 34 h after hCG administration, transabdominal follicular aspiration was performed using a variable frequency transvaginal transducer with ultrasound. A total of 71 oocytes were collected from 4 animals (average: 17). The meiotic maturity of oocytes was evaluated 3 h after retrieval. Ninety-one percent of oocytes were in metaphase 2 and of grades I and II which are appropriate for *in vitro* insemination.

**Key words:** Ovarian stimulation, GnRH agonist, ultrasound-guided oocyte retrieval, baboon

New protocols for superovulation and oocyte production have been developed over the last decade for assisted reproduction. One of the major advances has been the incorporation of gonadotropin releasing hormone agonists (GnRHa) to suppress pituitary control of ovarian function. The continued administration of GnRH as goserelin acetate, buserelin acetate or leuprolide acetate causes an initial

*The data were partly presented at the annual meeting of IETS in Maastricht, in 2000

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surge of gonadotropin release followed by long-term suppression of gonadotropin release as GnRH receptors are internalised (Meldrum, 1991). These procedures have been developed for human use and limited data are available to show the efficacy of these treatments in nonhuman primates.

In farm animals, GnRH or its analogues are used successfully in several fields of reproduction, such as oestrus synchronisation, treatment of cystic ovarian disease and stimulating follicular development, etc. The advantages of the use of GnRH in contrast to LH or hCG are: small molecular size, it is not likely stimulate an immune response, therefore repeated treatment with GnRH or its analogues does not result in refractoriness and is less expensive than that of LH or hCG (Brüssow et al., 1994; Youngquist, 1997).

Human and nonhuman primate (NHP) oocytes are generally retrieved by follicular aspiration before ovulation following controlled ovarian stimulation (COS) for assisted reproduction. Follicular aspiration in NHP has been performed by laparotomy or laparoscopy (Bavister et al., 1983; Bavister and Boatman, 1989; Clayton and Kuehl, 1984; Lopata et al., 1988; VandeVoort et al., 1989; Wolf et al., 1989; Wolf et al., 1990). Recently, successful transabdominal ultrasound-guided follicular aspiration has been reported in macaque (VandeVoort and Tarantal, 1991). However, no ultrasound-guided follicular aspiration has been published in baboon yet.

NHP gonadotropin preparations are not available, thus foreign gonadotropins (e.g. PMSG or human), usually human, are generally used to induce ovarian stimulation (Dukelow and Vengesa, 1986; Wolf et al., 1990). Human menopausal gonadotropin (menotropin, Pergonal®, Serono, Randolph, MA), a urinary extract containing equivalent amounts of FSH and LH activity, and human urofollitropin (Metrodin®, Serono, 1995) containing primarily FSH have been used for baboon COS (Fourie et al., 1987; McCarthy et al., 1991). No data are available on COS with GnRHa and hphFSH or rhFSH in baboon.

The objectives of this study were *(1)* to investigate whether baboon females respond to an ovarian stimulation protocol incorporating pituitary suppression with a GnRH agonist and either highly purified human FSH (hphFSH) or recombinant human FSH (rhFSH) with follicular development and oocyte maturation, and *(2)* to evaluate the effectiveness of using a transvaginal transducer for transabdominal ultrasound-guided oocyte retrieval in baboons.

## Materials and methods

### *Controlled Ovarian Stimulation*

A modified human ovulation induction protocol was applied to five adult female baboons (6 to 15 years of age, 12 to 17 kg) with a history of regular menstrual cycles (32 to 34 days) (Fig. 1). The animals were caged alone in a con-

trolled environment (25–27 °C, humidity 70%, 12/12 hour light/dark cycle). Their menstrual cycle was monitored by observation of perineal sex skin turgeses (follicular phase) and deturgeses (after ovulation). Cycle day 1 (CD 1) was the first day menses was observed. A long-acting GnRHa implant containing goserelin acetate (3.6 mg, Zoladex®, ICI Pharma, Wilmington, DE) was placed s.c. on CD 22–24 (luteal phase) of their menstrual cycle. Menses occurred about 10 days after GnRHa implantation. Following menses, on Days 3 and 6, the serum E2 levels were checked. Daily hphFSH (Fertinex™, Serono; 2 animals, 75 IU, i.m.) or rhFSH (3 animals, 75 IU, i.m.) were started ~ 10 days following menses. When the majority of follicles were > 5 mm in diameter and the E2 levels reached their maximum, 2000 IU hCG (Profasi®, Serono) was administered i.m. to induce final oocyte maturation and ovulation.

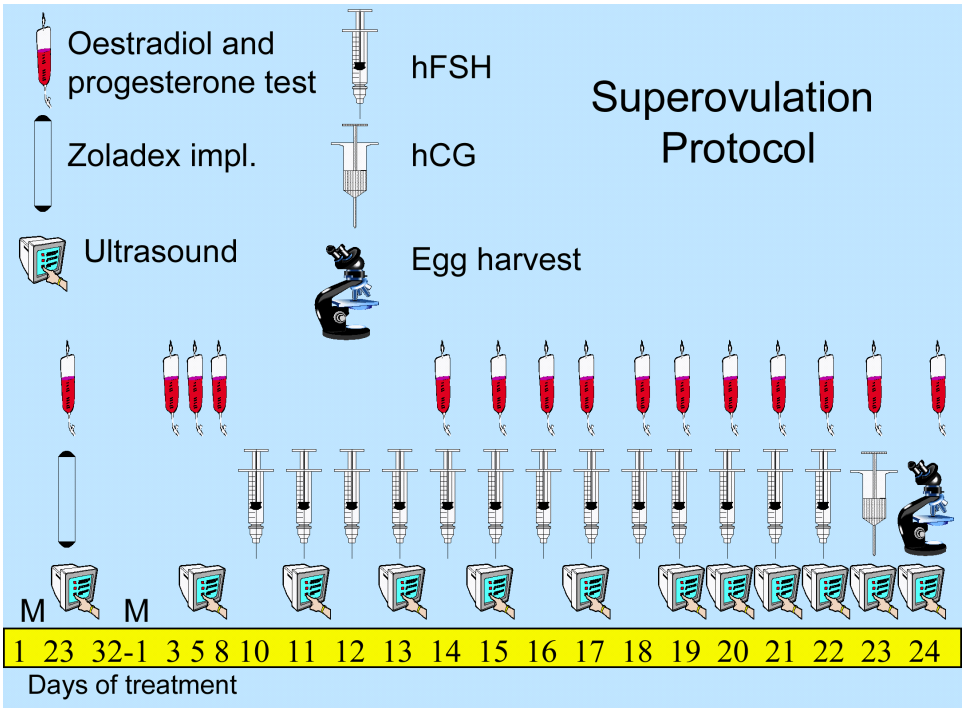


*Fig. 1.* Superovulation protocol

*Serum hormone test*

Non-isotopic methods were used to measure serum levels of oestradiol (E2), progesterone (P4) and hFSH. Serum E2, P4 and hFSH levels were obtained by immunoassay (ELISA) (DPC, Diagnostic Products Corporation, Los Angeles, USA). The inter-assay coefficient of variation over the calculated range of the assay did not exceed 8%.

*Egg retrieval*

Transabdominal follicular aspiration was performed 30 to 34 h after hCG administration using a variable frequency transvaginal transducer (Endo-V transducer, 5.0/6.0/7.5 MHZ; Siemens, Isaaquah, WA) with an ultrasound system (Siemens Sonoline SI-250). The follicular contents were aspirated into culture tubes using a disposable 17-gauge 30 cm long double-lumen needle connected to tubing (Swemedlab, Frounda, Sweden) and a suction pump (Rocket, Alpharetta, GA) set at 100 mm Hg. Follicles were rinsed with modified human tubal fluid (mHTF, Irvine Scientific, Irvine, CA) at 37 °C supplemented with 10% synthetic serum substitute (SSS, Irvine Scientific, Irvine, CA). Oocytes were assessed for meiotic maturity 3 h after retrieval. Oocytes were examined for presence of cytoplasmic vesicles and evidence of nuclear maturation. Only oocytes that had extruded a first polar body were considered appropriate for *in vitro* insemination.

## Results

Serum levels of P4 in all cases of COS were between 10.3 and 13.8 ng/mL at the beginning of the GnRHa treatments indicating that the animals were in the luteal phase which was suitable to start pituitary suppression. GnRHa pituitary suppression was evident by low E2 levels (< 20 pg/mL) and absence of perineal turgeses in the days just prior to FSH injection. Figure 2 shows the E2 concentrations in five baboon females during the COS cycles. The serum E2 levels in all COS cycles, except one, increased 4 to 17-fold to a peak around Days 9 and 12 post gonadotropin administration. E2 levels began to rise 5 to 7 days after beginning FSH treatment. Sonographic evidence of the follicular development was not observed transvaginally or transabdominally until treatment Days 9 to 10 when several follicles 2 to 3 mm in diameter and multiple smaller follicles became visible. The serum E2 reached plateau levels of 204, 207, 306 and 966 pg/mL between treatment Days 8 to 12. Follicle sizes increased to > 5 mm between treatment Days 9 and 12 and hCG was administered (Fig. 3).

Figure 4 shows the circulating human FSH concentrations detected in serum of four baboon females during COS beginning at the time of the first intramuscular injection of either 75 IU hphFSH or rhFSH. The values increased very rapidly during the first treatment day, then moderately rapidly. The serum hFSH reached plateau levels of 11 to 13 mIU between treatment Days 9 to 11.

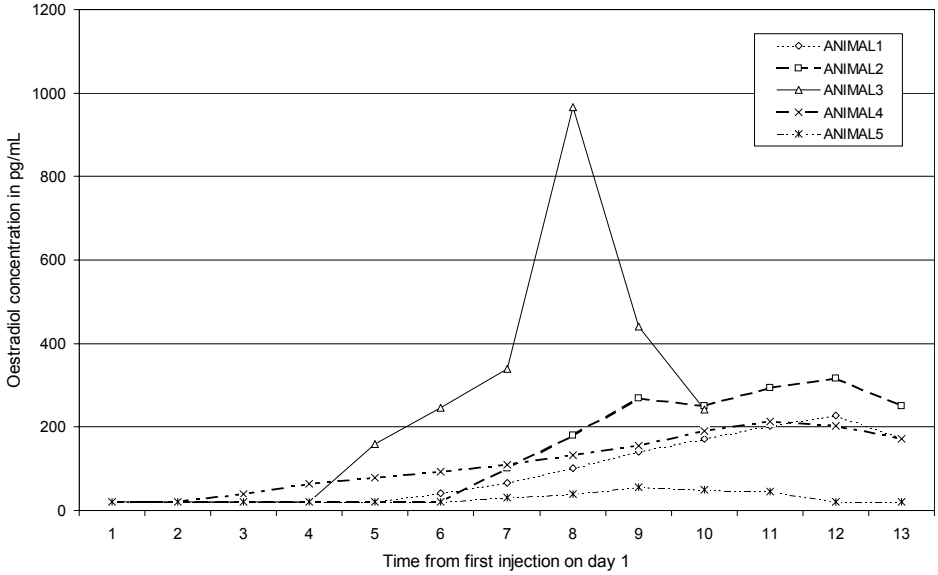


*Fig. 2.* Serum oestradiol concentrations in the five baboon females during controlled ovarian stimulation

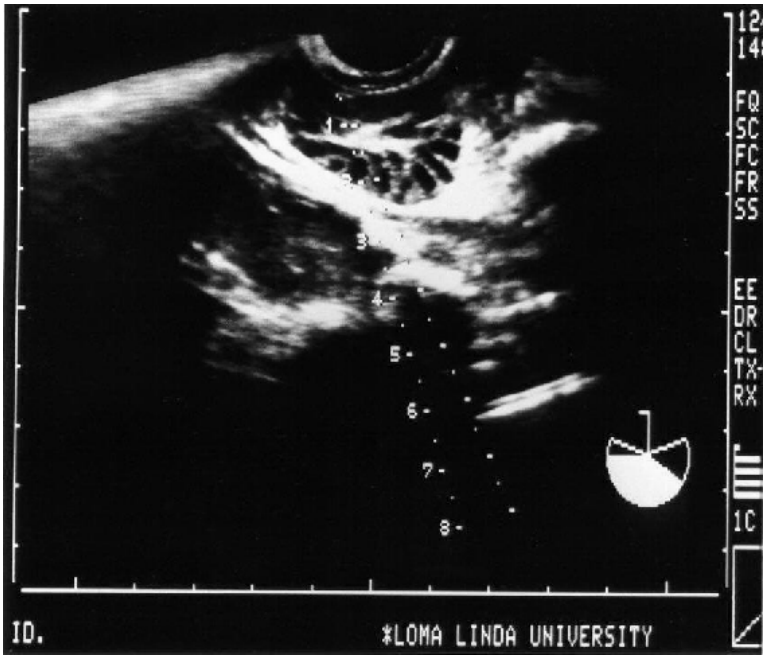


*Fig. 3.* Representative ultrasonogram of a baboon ovary containing multiple follicles, just before aspiration

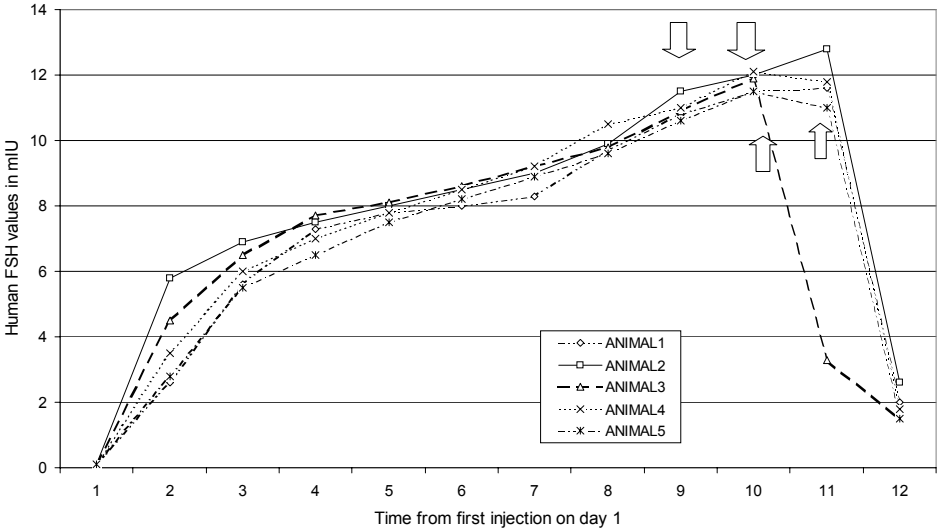


*Fig. 4.* Human FSH concentrations detected in serum during controlled ovarian stimulation of five baboon females

Follicular development was followed with ultrasonography. Follicular aspiration was carried out from 4 of five treated animals between 30 to 34 h after hCG injection. One animal did not respond to the treatment and the ovarian stimulation was cancelled. Fourteen, 21, 16 and 20 oocytes (n = 71) were collected from 4 animals. The average recovery rate of oocytes from the rinsed follicles was 86% (71/82). Ninety-one percent of the collected oocytes were morphologically normal, all in metaphase 2 and of grades I and II, which are considered appropriate for *in vitro* insemination. An average of 17 ova was obtained from each treatment cycle.

## Discussion

This study was undertaken to test the hypothesis that baboon females will respond with follicular development and oocyte maturation to a modified human controlled ovarian stimulation protocol incorporating a luteal phase start with a GnRHa followed by injection of either hphFSH or rhFSH. Our results demonstrate that a 3.6 mg Zoladex® implant provided sufficient GnRHa to suppress pituitary control of ovarian function in baboon females. As in the human female, initiation of GnRHa in the luteal phase prevents follicular development and E2 secretion following menses which was documented by the low serum level of E2 and the suppressed baboon perineal sex skin turgescence. Our data indicate that during GnRHa suppression, baboon females are able to respond to the human gonadotropin treatment. The circulating serum E2 levels increased 4 to 17-fold to

a peak and this elevation was sufficient to stimulate sex skin turgescence. The data indicate that highly purified human FSH and recombinant human FSH induce follicular development associated with E2 production in the baboon ovary. Our results show that 9 to 10 days injection of either 75 IU hphFSH or rhFSH per day produced peak E2. Daily injections of FSH resulted in a plateau of serum values by 8 to 9 days of treatment. A similar pattern has been observed in women treated with daily injections of hFSH (Porchet et al., 1994).

In this preliminary study, ova were used for a variety of pilot experiments such as intracytoplasmic sperm injection (ICSI). Using ICSI, we could demonstrate that the collected oocytes are capable of fertilisation. This indicates that sufficient exposure to *in vivo* conditions was provided and the quality of ova obtained appropriate for *in vitro* fertilisation.

In summary, highly purified human FSH or recombinant human FSH effectively induce COS and oocyte development in the baboon suppressed with GnRHa. The variations in hormone profiles during ovarian stimulation were similar to those found in humans, but the E2 levels measured were lower. The depot form of GnRHa is effective and beneficial for baboons because the requirement of daily animal access for administration by injection is minimised. Transabdominal ultrasound-guided follicular aspiration using a transvaginal transducer is effective and safe for oocyte retrieval in baboon. Morphologically normal mature oocytes in metaphase 2 and of grades I and II can be obtained and they are capable of fertilisation.

## Acknowledgements

The authors are grateful to Thomas J. Kuehl Ph.D. (A & M University, College of Medicine) for providing helpful discussion during this project. We are also grateful to Serono Laboratories for providing the recombinant human FSH to the experiments. We are very thankful to Katy Brasky DVM (Southwest Foundation for Biomedical Research) for her help. We wish to thank Geoffrey Visbal, Nancy Holmqvist, Joyce Ivy and Jan Smallwood for excellent technical assistance. We also wish to thank Evelyn Agcaoli, Lane Beha and Simran Bal Toor for conducting the ELISA.

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