The Effect of Human Urinary Luteinizing Hormone (LH) Inhibitor on Implantation and Pregnancy in the Rat (34820)

A. JAGANNADHA RAO, M. R. SAIRAM, H. G. MADHWA RAJ, AND N. R. MOUDGAL
(Introduced by Prof. Roy O. Greep)
Endocrinology Laboratory, Department of Biochemistry, Indian Institute of Science, Bangalore-12, India

Though several reports have appeared regarding the presence of a LH inhibitor in human urine (1–4), Hahn and Albert (5) and Hipkin (6) using the kaolin–acetone method of Albert were unable to obtain an active preparation from human urine. Moudgal *et al.*, (7) while confirming the ineffectiveness of the kaolin–acetone procedure to prepare active extracts, have recently observed that extraction of urine by the method of Futterweit *et al.* (3) does give an active preparation. Most workers have used the inhibition of HCG (human chorionic gonadotropin)-induced uterine weight increase, in immature female mice, as an index to assay the inhibitor activity. Ota *et al.* (8, 9) have tested the specificity of the inhibitor preparation by using it to inhibit HCG-induced superovulation in mice. After our recent observation that neutralization of endogenous luteinizing hormone (LH) with LH antiserum in pregnant rats during the pre- and postimplantation periods up to Day 12 leads to inhibition of implantation and termination of gestation (10–12), it was considered useful to test the specificity of the human urinary LH inhibitor in the above system.

Methods. Urine collected from healthy male volunteers between 8 AM–5PM daily (thymol was used as a preservative) was processed immediately after the collection period according to methods described earlier (7). Both kaolin–acetone method as described by Bradbury *et al.* (13) and a slightly modified kaolin–alcohol (3) method were used to obtain different urinary preparations.

The kaolin–alcohol method used in our laboratory essentially consists in adjusting the pH of the filtered urine to 5.0 with glacial acetic acid, adding to it a 20% suspension of acid-washed kaolin (pH of the suspension adjusted to be between 6 and 7 by repeated washing with 1 *N* HCl) and stirring mechanically at room temperature for 30 min and storing in cold overnight. Ten grams of kaolin were added per liter of urine. Next day the kaolin suspension was centrifuged, the precipitate washed with distilled water adjusted to pH 5.0 with acetic acid, and the inhibitor eluted from kaolin with 2 *M* ammonium hydroxide (30 ml per liter of urine). The eluate was adjusted to pH 8.5 with acetic acid, and the sediment obtained was discarded. The supernatant fluid was cooled to 0° and pH once again adjusted to 5.0 with acetic acid. The inhibitor was precipitated by addition of 5 vol of 95% alcohol also precooled to 0°. The precipitation was allowed to be completed for 48 hr in the cold, centrifuged, and the precipitate washed twice with cold acetone and ether and air-dried. This represented the crude gonadotropin inhibitor preparation (GIM) and the yield ranged from 20–40 mg/liter of urine.

In order to demonstrate the inhibitory activity of the urinary preparations, it was essential that the accompanying gonadotropic activity of the saline extract of the crude material be destroyed by heating in a boiling water-bath for 1 hr followed by centrifugation at 6000 rpm for 20 min. The supernatant fluid was used as the test inhibitory material. The HCG inhibitory activity of the preparation was initially checked by mouse uterine weight assay and the seminal vesicle response assay as described by us earlier (7). The crude extract prepared according to the

TABLE I. Effect of Human Urinary LH Inhibitor on Implantation and Maintenance of Gestation in the Rat.[a]

Group	Treatment[b]	Duration of treatment (days)	*N*	Laparotomy on Day 8 (T/NT)	Remarks
I	Saline	1–7	6	6/36	Littered normally on Day 23; average litter size 6
II	GIM (15 mg)	1–7	6	0/6	No implantation sites on Day 8 and no sites at autopsy on Day 22
III	GIM (50 mg)	8–9	8	7.3/59	Profuse bleeding on Day 10; all sites had resorbed by autopsy on Day 22
IV	KA (50 mg)	8–10	6	7.5/45	Delivered normally on Day 23; average litter size 7
V	Normal human serum (0.5 ml)	8–10	3	7.6/23	Delivered normally on Day 23; average litter size 7
VI	GIM (50 mg)	13–14	5	7.5/45	Delivered normally on Day 23; average litter size 7
VII	GIM (50 mg) + LH (100 μg)	8–9	6	7.1/42	Delivered normally on Day 23; average litter size 6

[a] *N* = number of rats per group; T = average number of sites per animal; NT = total number of sites.

[b] GIM. Urinary extract prepared according to the kaolin–alcohol method of Futterweit *et al.* (3). KA. Urinary extract prepared according to the kaolin–acetone method of Albert according to Bradbury and Brown (13). GIM and LH were administered at different sites. The gonadotropic activity of both GIM and KA used here was destroyed by heat inactivation as described in the text.

kaolin–acetone procedure, as mentioned earlier, had no inhibitory activity.

Colony-bred female rats weighing 120–150 g were caged with proved males and the day sperm was found in the vagina was considered as Day 1 of pregnancy. Laparotomies were performed on Day 8 of pregnancy and autopsy on Day 22. The inhibitor was administered on different days by subcutaneous route in saline, and LH was given in 16% gelatin. Appropriate control groups were included which received saline, inactive kaolin–acetone preparation (KA), and normal human serum, respectively. The nature and number of implantation sites were noted at laparotomy and autopsy.

Results and Discussion. It is apparent from Table I that administration of the active inhibitor preparation (GIM) during the pre- and postimplantation periods results in the inhibition of implantation and termination of pregnancy (Groups II, III). It should be noted that none of the control groups (Groups I, IV, and V) showed any inhibition demonstrating that it is not due to nonspecific effects. This was further confirmed by the fact that GIM had no effect when administered on Days 13 and 14 of pregnancy (Group VI). This is similar to the observed lack of effect of LH antiserum administered after Day 12 of pregnancy (12). Finally, the fact that LH was able to reverse the inhibition brought about by GIM (Group VII) points to the specific action of the inhibitor.

Thus, this investigation, by confirming our observation with the effects of LH antiserum on the first 12 days of pregnancy in the rat, not only affirms the essential need for LH during this period, but also strongly supports the contention that the human urinary extracts do possess an LH inhibitor. Attempts are presently being made in our laboratory to further purify and characterize this inhibitor material.

Summary. The presence of an LH inhibitor in human urine has once again been confirmed by testing the ability of the preparation to inhibit implantation and terminate gestation in intact pregnant rats.

The authors are grateful to the Ford Foundation, New York, and the Ministry of Health and Family Planning, Government of India, New Delhi, for financial assistance.

1. Soffer, L. J., Futterweit, W., and Salvanseschi, J., J. Clin. Endocrinol. Metab. **21**, 1267 (1961).

2. Soffer, L. J., Salvanseschi, J., and Futterweit, W., J. Clin. Endocrinol. Metab. **22**, 532 (1962).

3. Futterweit, W., Margolis, S. A., Soffer, L. J., and Dorfman, R. I., Endocrinology **72**, 903 (1963).

4. Soffer, L. J., and Fogel, M., J. Clin. Endocrinol. Metab. **24**, 651 (1964).

5. Hahn, H. B., Jr., and Albert, A., J. Clin. Endocrinol. Metab. **25**, 409 (1965).

6. Hipkin, L. J., J. Endocrinol. **38**, 39 (1967).

7. Moudgal, N. R., Sairam, M. R., and Madhwa Raj, H. G., Gen. Comp. Endocrinol. Suppl. **2**, 162 (1969).

8. Ota, M., Dronkert, A., and Gates, A H., Endocrinol. Japonica **14**, 289 (1967).

9. Ota, M., Obava, K., and Dronkert, A., Endocrinol. Japonica **14**, 308 (1967).

10. Madhwa Raj, H. G., Sairam, M. R., and Moudgal, N. R., J. Reprod. Fert. **17**, 335 (1968).

11. Moudgal, N. R., Madhwa Raj, H. G., Jagannadha Rao, A., and Sairam, M. R., Indian J. Exp. Biol. **7**, 45 (1969).

12. Madhwa Raj, H. G., and Moudgal, N. R., Endocrinology **86**, 874 (1970)

13. Bradbury, J. T., Brown, E. S., and Brown, W. E., Proc. Soc. Exp. Biol. Med. **71**, 228 (1949).

Received Feb. 24, 1970. P.S.E.B.M., 1970, Vol. 134.