Ann Rech Vet 1987 18: 57-61

CHANGES IN OVARIAN FUNCTION ASSOCIATED WITH OXYTOCIN INJECTION TO IMMATURE RATS

AL-JANABI AS[1] and YOUSIF WH[2]

1: State Board for Applied Agricultural Research, Baghdad, Iraq
2: College of Veterinary Medicine, Mousil University, Iraq

Received, 25 June, 1985/Revised Version, 20 May, 1986/Accepted, 5 June 1986

Résumé

MODIFICATIONS DES FONCTIONS OVARIENNES APRÈS INJECTION D'OCYTOCINE À DES RATTES IMMATURES — Nous avons recherché chez la ratte impubère âgée de 25 jours, les effets de l'ocytocine sur la fonction ovarienne. L'hormone est administrée par voie sous-cutanée pendant cinq jours consécutifs aux doses de 250 ou 50 mμ/animal à des rattes hysterectomisées ou non, ou recevant simultanément de l'indométhacine (2 mg/kg). Le poids des ovaires et de l'utérus avait augmenté chez tous les animaux traités ainsi que les taux circulants d'œstradiol 17β. Le taux de progestérone n'était pas modifié. L'intérêt de ce travail est de préciser le rôle trophique de l'ocytocine sur l'ovaire et l'utérus et d'en étudier le mécanisme. La prostaglandine E ne semble pas intervenir dans le mode d'action.

Although the isolation of oxytocin from the posterior pituitary was achieved more than half a century ago (Kamm *et al* 1928), its real role in controlling several reproductive phenomena remains obscure. Concerning the role of oxytocin in gonadotropins secretion, for instance, available informations are either unclear or contradictory. Martini *et al* (1959), for example, observed a sharp increase of urinary gonadotropins in female rabbits after pitocin or synthetic oxytocin injection. Also Melin (1971) reported that injected oxytocin is capable of reducing total pituitary gonadotropins level to about 20 % of the control in male rabbits. On the other hand, several other authors (Wilks and Hansel 1971, Vaughan *et al* 1979) reported that oxytocin had no significant effect on the hypophyseal levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in heifers and castrated adult male rats. With respect to ovarian function, several investigators (Armstrong and Hansel 1959, Carlson and Black 1969, Milvae and Hansel 1980) have reported that oxytocin injection can shorten estrous cycle probably by virtue of its ability to reduce corpus luteum function. In the present investigation, an effort has been made to study the effects of oxytocin on growth of ovaries and uteri, then on hormonal production. It was shown that circulating levels of estradiol 17 β increased while progesterone levels did not change.

Materials and Methods

Oxytocin was administered in two doses: 50 (a) or 250 (b) mμ per day per animal.

1. *Experimental groups*

group 1: rats treated with oxytocin only (20 rats): 10 (dose a) and 10 (dose b).
group 2: rats treated with oxytocin and indomethacin (19 rats): 9 (dose a) and 10 (dose b).
group 3: hysterectomized rats (10 rats).
group 4: sham hysterectomized rats (10 rats).
group 5: rats receiving saline, either injected subcutaneously (10 rats) or administered orally (10 rats).

All animals were sacrified 24 hours after the last injection. Ovaries and uteri were quickly removed, freed from fat tissues and thoroughly examined before weighting.

Blood was collected for hormonal assay (estradiol 17 β and progesterone).

Additionaly, 50 rats treated only with oxytocin: 25 rats (dose a) and 25 (dose b) and 20 control rats (injected subcutaneously with saline) were sacrified daily starting 24 hours after the beginning of injection to follow blood plasma level of estradiol 17 β and progesterone during five days.

2. *Animals*

In all experiments, immature female Sprague-Dawley rats were used. They were the offsprings of adult rats obtained from the colony of the Samara Drug Industries (SDI) in Iraq. At the age of 25 days (day of birth is day one), animals were randomly selected and kept under controlled conditions at room temperature of 25 ± 2 °C and a lighting schedule of 14 hours and 10 hours of darkness. Commercial pelleted diet (Iraq animals food establishment, Baghdad) and water were given *ad libitum*.

3. *Chemicals*

Oxytocin used was Synthocin R (Sandoz, ltd, Switzerland). The hormone was injected subcutaneously daily for five days in two doses of 50 and 250 mμ per animal.

Table 1. – Effect of oxytocin treatment on ovarian and uterine weight in intact and in indomethacin treated immature rats on day 6

treatment	rats (no.)	ovary (mg/100g BW)	uterus	
			wet weight (mg/100g BW)	dry weight (mg/100mg UW)
control (saline)	10	16.4 ± 0.4	58.5 ± 3.2	8.4 ± 0.4
oxytocine (mμ/rat/day)				
50	10	18.3 ± 0.2 a	72.4 ± 2.7 a	11.5 ± 0.7 a
250	10	18.7 ± 0.3 a	69.8 ± 1.6 a	11.3 ± 0.4 a
indomethacin and oxytocin (mμ/rat/day)				
50	9	19.4 ± 0.5 a	70.8 ± 4.5 a	10.8 ± 0.6 a
250	10	19.3 ± 0.6 a	75.4 ± 3.7 a	11.7 ± 0.9 a

a:significant at 0.05

Indomethacin used was Indocid R (Charles E Frost, USA). In all cases, the drug was administered orally using a stomach tube and at dose level of 2mg/kg.

4. *Samples*

Blood was collected through cardiac puncture, using heparinized tubes; plasma was then separated and stored at – 20 °C for the hormonal assay.

5. *Hormonal assay*

Estradiol 17 β and progesterone levels were measured using radioimmunoassay following the methods of Abraham *et al* (1971) and Malvano *et al* (1974) and using kits provided by International CIS (France).

6. *Statistical analysis*

All results were statistically analysed used Student t test (Steel and Torrie 1960).

Mean were presented with standard error.

Results

1. *Effects of oxytocin on uteri and ovaries*

Injection of oxytocin (group 1) caused a significant increase ($P \leqslant 0.05$) in both ovarian and uterine (wet and dry) weights, in comparison with the controls. A similar increase ($P \leqslant 0.05$) in both ovarian and uterine weights was also seen in animals treated with oxytocin and indomethacin at the same time (group 2), illustrated in table 1.

Hysterectomized animals with oxytocin showed a marked increased in ovarian weight (table 2), as compared to the sham hysterectomized ones. This increase was statistically significant ($P \leqslant 0.05$).

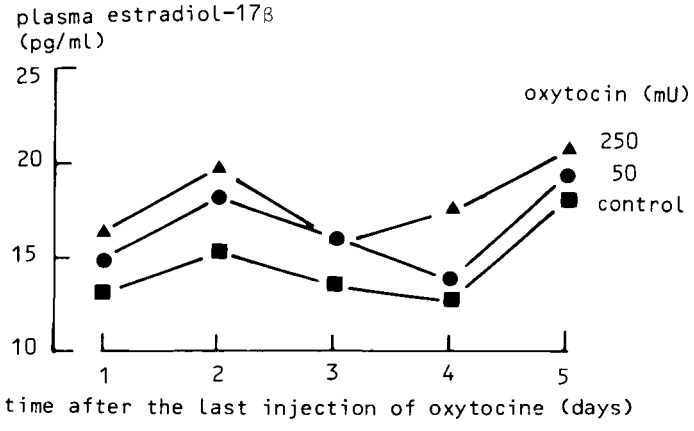

Fig 1 — Variations in plasma estradiol 17 β level during treatment for five days with 50 and 250 mμ of oxytocin. Each point represent the mean of five animals for the treated group and four animals for control

2. *Variations of plasma estradiol 17 β and progesterone*

Daily variations in plasma estradiol 17 β .evel are shown in figure 1. In rats injected with oxytocin, estradiol 17 β level remained higher than the control. In animals injected with 250 mμ of oxytocin, the level of the hormone showed a marke increase in comparison with 50 mμ oxytocin treated rats.

When animals were killed on day 5, a non significant increase in plasma estradiol 17 β was seen in animals treated with indomethacin and oxytocin in comparison with the control (fig 2).

A similar increase in plasma estradiol 17 β level was seen in hysterectomized animals treated with oxytocin in comparison with the sham hysterectomized ones (fig 3).

Plasma progesterone level, on the other hand, showed very little changes in treated animals in comparison with controls.

Discussion

The general trend of our results seems to agree with results reported previously by other authors. Corbin and Shottelius (1961) reported that injection of oxytocin accelerated vaginal canalization and caused premature reproductive organs growth in rats. Ovarian growth in rats (Malven and Hansel 1965) and increase in urinary estrogen secretion in cows (Lunaas 1973) was also reported after oxytocin injection. A similar study to ours (Al-Hussary and Al-Janabi 1979) reported ovarian changes in immature mice almost identical to results reported in this communication. In view of the fact of absence of clear picture concerning changes in gonadotropins levels (LH and FSH) in our investigation, it is rather difficult to indicate for certain how this changes were brought about. However examining available literature may suggest following possibilities:

1. Injected oxytocin could have acted on the hypothalamus stimulaling the release of gonadoliberine, thus promoting the synthesis and/or the release of gonadotropins (especially LH and FSH) from the anterior pituitary, or it could have behaved itself as a releasing hormone for gonadotropins. There is no direct support for either of these theories. Although Martini *et al* (1968) in an attempt to demonstrate a relationship between oxytocin and gonadotropins release, reported that oxytocin and gonadotropin releasing hormones are both found in the paraventricular nucleus. Also Hansel and Wagner (1960) reported that oxytocin of hypothalamic origin may be involved in some

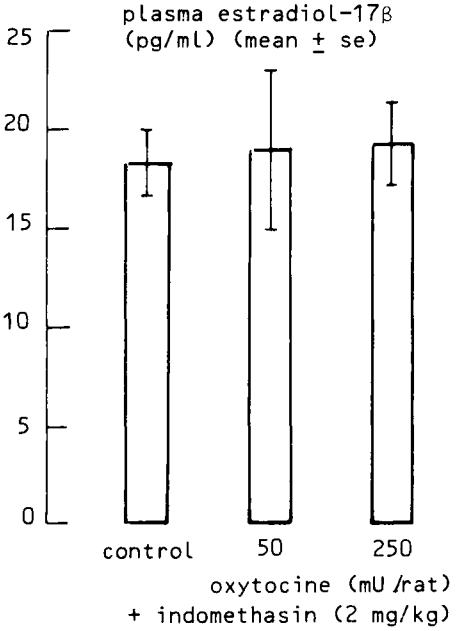

Fig 2 — Effect of treatment for five days with 50 and 250 mμ of oxytocin together with indomethacin on plasma estradiol 17 β level on day 6

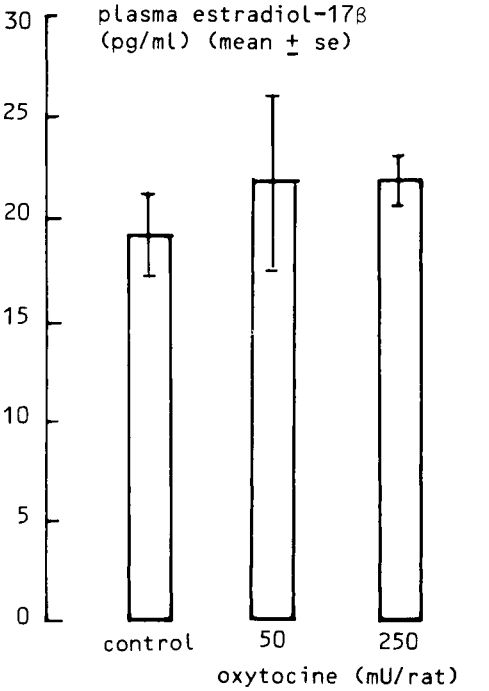

Fig 3 — Effect of treatment for five days with 50 and 250 mμ of oxytocin on plasma estradiol 17 β level in hysterectomized immature rats on day 6.

Table 2. – Effect of oxytocin treatment on ovarian weight in hysterectomized immature rats on day 6

treatment	rats (no.)	ovary (mg/100g BW)
control	10	16.1 ± 0
oxytocin (mμ/rat/day)		
50	10	21.0 ± 0.7 a
250	10	20.3 ± 0.9 a

a:significant at 0.05

way in the regulation of pituitary gonadotropin secretion. Moreover several authors reported that oxytocin reduced total pituitary gonadotropins content by 20 % in rabbit (Melin 1971) and about 50 % in heifers during estrous or on day 7 of the cycle (Donaldson *et al* 1965). However other authors found that oxytocin had no effect on concentration of LH in heifers (Wilk and Hansel 1971) or in female rats (Shani *et al* 1976).

2. Injected oxytocin may have acted on the ovary inducing ovarian growth and steroidogenesis. Results available in recent literature seems to disagree with this assumption. Since it has been shown that oxytocin has an inhibitory effect on steroid synthesis in ovarian and testicular cells in culture (Tan *et al* 1981, Adashi and Hsueh 1981), although Tan *et al* (1982) suggested that oxytocin in low concentration may stimulate progesterone by bovine luteal cells.

It is rather difficult to favor any of the above possibilities in view of the fact that gonadotropins picture (in our investigation) in blood during oxytocin administration and other treatments is lacking.

However, failure of hysterectomy and indomethacin treatments to alter ovarian response to oxytocin injection seems to eliminate the possibility that changes obtained were associated with prostaglandin E secretion. We are inclined, however, to favor the possibility of stimulation of gonadotropins secretion due to oxytocin injection. Subsequent studies involving determination of different hypothalamic and pituitary hormones concerned, may help in throwing more light on the actual route of effect.

Summary

The effects of oxytocin on ovarian function have been studied in immature rats of 25 days of age. The hormone was injected subcutaneously daily for 5 days in two doses: 50 and 250 mμ per animal, to intact females, hysterectomized or injected simultaneously with indomethacin (2 mg/kg). Ovarian and uterine weights increased in all treated animals. Plasma estradiol 17 β levels increased, while plasma progesterone level did not change. The relevance of this study was to precise the trophic role of oxytocin on ovary and uterus. The changes observed did not seem to be associated with prostaglandin E secretion.

References

ABRAHAM GE, SWERDLOFF R, TULCHINSKY D, ODELL WD, 1971. Radioimmunoassay of plasma progesterone. J Clin Endocrinol 32:619-624

ADASHI EY, HSUEH AJW, 1981. Direct inhibition of testicular androgen biosynthesis revealing antigonadal activity of neurohypophysial hormones. Nature 293:650-652

AL-HUSSARY NA, AL-JANABI AS, 1979. Oxytocin, new effect: ovulation induction in immature mice. J Steroid Biochem 2:XVI-XVII

ARMSTRONG DT, HANSEL W, 1959. Alteration of the bovine estrous cycle with oxytocin. J Dairy Sci 42:533-542

CARLSON J, BLACK DL, 1969. Oxytocin produced changes in the bovine ovary before and after unilateral ovarectomy. J Reprod Fert 20:39-43

CORBIN A, SCHOTTELIUS BA, 1961. Hypothalamic neurohormonal agents and sexual maturation of immature female rats. Am J Physiol 201:1176-1180

DONALDSON LE, HANSEL W, VANVLECK LD, 1965. Luteotrophic properties of luteinizing hormone and nature of oxytocin induced luteal inhibition in cattle. J Dairy Sci 48:331-337

HANSEL W, WAGNER WC, 1960. Luteal inhibition in the bovine as a result of oxytocin injections, uterine dilation, and intrauterine infusion of seminal and preputial fluids. J Dairy Sci 43:796-805

KAMM O, ALDRICHT TB, GROTE IW, ROWE LW, BUGBEE EP, 1928. The active principles of the posterior lobe of the pituitary gland. J Am Chem Soc 50:573-601

LUNAAS T, 1973. Urinary œstrogenes in cows treated with oxytocin during metestrus. Res Vet Sci 14:124-126

MALVANO R, TROISI L, GANDOLFI C, ATTANASIO A, OROSIGNANI PG, 1974. Radioimmunoassay of some gona-

dal steroids: validity and limitations of measurements in plasma extracts. In Crosignani PG, James VHT, eds, Recent progress in reproductive endocrinology. Academic Press, London

MALVEN PA, HANSEL W, 1965. Effect of bovine endometrial extracts, vasopressin and oxytocin on the duration and pseudopregnancy in hysterectomized and intact rats. J Reprod Fert 9:207-215

MARTINI L, MIRAL L, PECLE A, SAITO S, 1959. Neurohypophysial hormones and release of gonadotropins. J Endocrinol 18:245-250

MARTINI L, FRASCHINI F, MOTTA M, 1968. Neural control of anterior pituitary functions. Recent Prog Horm Res 24:439-496

MELIN P, 1971. Spermatogenesis and sperm output in rabbits after long term treatment with oxytocin. Acta Endocrinol 66:515-528

MILVAE RA, HANSEL W, 1980. Concurrent uterine venous and ovarian arterial prostaglandin F concentrations in heifers treated with oxytocin. J Reprod Fert 60:7-15

SHANI J, URBACH L, TERKEL J, GOLDHABER G, 1976. Serum prolactin and LH in chronically annulated cycling rats after intra-atrial administration of oxytocin. Arch Int Pharmac Dyn 221:323-327

STEEL RGD, TORRIE JE, 1960. Principles and procedures of statistics. McGraw-Hill, New York

TAN GJS, TWEEDALE R, BIGGS JSG, 1981. Oxytocin may have a role in the regulation of the human corpus luteum. J Steroid Biochem 14:XII

TAN GJS, TWEEDALE R, BIGGS JSG, 1982. Effects of oxytocin on the bovine corpus luteum of early pregnancy. J Reprod Fert 66:75-78

VAUGHAN MK, BLACK DE, JOHON LY, REITER RJ, 1979. The effect of subcutaneous injections of melatonin, arginine, vasotocine and related peptides on pituitary and plasma levels of luteinizing hormones follicle stimulating hormone and prolactine in castrated adult male rats. Endocrinology 104:212-217

WILKS JW, HANSEL W, 1971. Oxytocin and the secretion of luteinizing hormone in cattle. J Anim Sci 33:1048-1052