

EFFECT OF PROGESTERONE AND OXYTOCIN ON THE PITUITARY–OVARIAN RELATIONSHIP IN HEIFERS

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Summary. The effects of oxytocin and of progesterone on the pituitary–ovarian relationships were studied in sixteen non-pregnant heifers. Subcutaneous injection of 100 mg of progesterone in corn oil daily for 35 days from Day 7 of the oestrous cycle depressed both the size of the largest follicle and the total weight of follicular fluid. The FSH level was significantly raised after progesterone injections as compared to Day 1 of the cycle but not as compared to Day 7. The progesterone treatment had no significant effect on LH level. It was concluded that progesterone curtails the production, and consequently the release, of FSH and LH.

The injection of 150 U.S.P. units of oxytocin daily from the day of oestrus to Day 6 of the cycle produced a smaller corpus luteum (2.51 versus 4.40 g, $P < 0.05$) and a reduced concentration of progesterone (14.6 versus 29.0 $\mu\text{g/g}$, $P < 0.05$). The oxytocin treatment had no significant effect on the hypophyseal levels of FSH and LH. It did not affect the size of the largest follicle or the total follicular fluid weight.

The corpus luteum of the previous cycle, still present on the day of oestrus, was small (1.74 g) but contained a detectable amount of progesterone (2.8 $\mu\text{g/g}$).

INTRODUCTION

Progesterone and oxytocin have marked effects on the oestrous cycle; the former blocks oestrus and the latter induces oestrus and ovulation precociously (Armstrong & Hansel, 1959). Several attempts have been made to characterize the effect of progesterone at the pituitary level [Foote, Waldorf, Self & Casida (1958) and Rigor, Meyer, First & Casida (1963) in the pig; Bellows, Pope, Meyer, Chapman & Casida (1963) in the sheep; Ray, Emmerson & Melampy (1961) in the cow and Van Rees (1959a, b), Rothchild (1962) and Bellows (1962) in the rat]. It appears that progesterone administration during the oestrous cycle increases gonadotrophin potency in the rat and pig, whereas in ruminants it either has no effect (sheep) or depresses the gonadotrophic potency (cows). However, the long-term effects of progesterone treatment have been little studied in the larger species, particularly the cow. Hansel & Wagner (1960)

found that either treatment with oxytocin or treatments which induce release of oxytocin, such as uterine dilatation, shorten the length of the cycle from 21 to about 8 to 12 days in heifers. One of the possibilities suggested by these authors is that oxytocin causes release of excessive gonadotrophin other than luteotrophin from the pituitary gland, resulting in lysis of the corpus luteum.

The present study had a dual purpose: (1) to characterize at the pituitary and ovarian levels the long-term effects of daily injections of progesterone and (2) to determine whether oxytocin, in doses large enough to decrease corpus luteum size, has any effect on pituitary gonadotrophic potency or follicular growth.

MATERIALS AND METHODS

The experiment involved four groups, each of four sexually mature heifers. The animals in Groups I and II were slaughtered on Day 1 (day of oestrus and Day 7 of the oestrous cycle, respectively). Group III heifers were injected subcutaneously with 150 units of oxytocin (Norden Laboratories) daily from Day 1 to Day 6 of the cycle and slaughtered on Day 7. Group IV heifers received 100 mg of progesterone daily in corn oil from Day 7 to Day 41 and were slaughtered on Day 42. All of the animals were checked for heat twice daily and the ovaries of the animals receiving progesterone were palpated *per rectum* weekly throughout the experimental period.

At the time of slaughter the pituitary glands and ovaries were collected from all the animals. The ovaries from each animal were weighed, and the corpus luteum, when present, was enucleated, weighed and stored in 95% ethanol for determination of progesterone content. The largest follicle was aspirated with a syringe and its volume determined. The ovaries were then sliced so as to rupture the visible vesicular follicles and rolled on moistened absorbent paper. The ovarian stroma was then re-weighed. The difference between the first and the last weight minus the corpus luteum weight gave the total follicular fluid weight, which was used as an additional measure of follicular growth.

The pituitary glands were dissected free of connective tissue and the posterior lobe was removed. The weight of the anterior lobe was recorded and the glands were stored frozen. At a later date the glands were homogenized, lyophilized and tested individually for FSH and LH activity.

A modification of the method of Steelman & Pohley (1953) was used to measure pituitary FSH. The modifications involved: (1) using 26-day-old instead of 21- or 22-day-old rats, (2) injecting for 5.5 days twice daily and killing the rats 24 hr later instead of injecting them three times daily for 3 days and killing on the 4th day, and (3) using an augmentor in addition to the 20 i.u. of HCG; 5.5 mg of haemin dissolved in a weak solution of sodium hydroxide was added to each dose.

A single level of 75 mg of pituitary powder per rat (total dosage) was injected. Two rats per gland were employed. The mean ovarian weight of these two rats constituted the response. The tests were performed in four replicates, each replicate consisting of one gland from each treatment group. Glands from each treatment group were assigned to a particular replicate at random. Two

groups of control rats (one receiving 20 i.u. of HCG alone and the other 20 i.u. of HCG with 5.5 mg of haemin) were also run with each replicate.

The percentage ovarian ascorbic acid depletion as measured by the method of Parlow (1958) was used as an index of pituitary LH. Three doses (0.5, 1.0 and 1.5 mg of pituitary powder) with two rats per dose level were employed. A control ovary was removed from each rat before injecting the given dose. All the injections were made through a tail vein in 1 ml of saline. The 'treated' ovary was removed 4 hr \pm 10 min following the injection. Ascorbic acid determinations were made by a modification of the method of Mindlin & Butler (1938). Progesterone was determined by the method of Loy, McShan & Casida (1957) as modified by Zimbelman (1960).

Statistical analysis of the data was performed by analysis of variance. Whenever more than two means were involved Duncan's Multiple Range Test (Steel & Torrie, 1960) was used to determine the significant comparisons. In the pituitary studies, replicate-treatment interaction was used as an error term to test the significance of treatment effects.

RESULTS

Corpora lutea

None of the heifers in the progesterone-treated group came into oestrus during the experimental period. No ovulations were detected by palpation of the ovaries during the treatment period, and at the time of autopsy the ovaries of these animals contained no new corpora lutea. The treatment was therefore effective in blocking heat and ovulation during the experimental period.

The oxytocin treatment resulted in significantly smaller corpora lutea (2.51 versus 4.40 g, $P < 0.05$, Table 1). The corpora lutea from this group had cavities containing blood clots. None of the corpora lutea from the control (Day 7) group contained blood-filled cavities although some had cavities containing clear fluid. The latter condition seems to be normal at this stage of development (Hansel, 1959). The Day 7 group had significantly larger corpora lutea than the Day 1 group (4.40 versus 1.74 g, $P < 0.01$, Table 1). The corpora lutea present in the latter must have survived from the previous oestrous cycle since none of the animals in this group had reovulated.

Analysis of progesterone concentration disclosed a highly significant effect of oxytocin treatment. The 'oxytocin-treated' corpora lutea contained approximately half as much progesterone as Day 7 control corpora lutea (14.6 versus 29.0 $\mu\text{g/g}$, $P < 0.01$, Table 1). Although the 'old' corpora lutea on the day of oestrus were small (Table 1), they still contained a detectable amount of progesterone (2.8 $\mu\text{g/g}$).

Volume of the largest follicle and total follicular fluid weights

The largest follicles present in the progesterone-treated group were significantly smaller than those found on the day of oestrus (0.6 versus 1.8 ml, $P < 0.05$, Table 1). The oxytocin treatment had no significant effect on the size of the largest follicle when compared with the Day 7 group (Table 1). The stage of the oestrous cycle also had no significant effect on this parameter although

there was a tendency for the Day 1 group to have larger follicles than the Day 7 group (1.8 versus 1.1 ml, Table 1).

The progesterone treatment caused a significant depression in total follicular fluid weight (Table 1). The mean follicular fluid weight was greatest in the oxytocin-treated group; but this was not significantly different from that found in Day 7 control animals (7.11 versus 6.32 ml, Table 1). Stage of the cycle had no significant effect on the total follicular fluid weight (Table 1).

Pituitary studies

Administration of haemin along with HCG in the FSH tests did not augment the ovarian weight of the rat as compared to treatment with HCG alone (53 versus 51 mg). Analysis of the ovarian weights of the test rats disclosed a significant effect of treatment of the heifers (Table 1). The progesterone-treated

TABLE 1
INFLUENCE OF OXYTOCIN, PROGESTERONE AND STAGE OF OESTROUS CYCLE ON VARIOUS PARAMETERS

Parameter	Group				Error mean square†
	Day of oestrus (Day 1)	Day 7 of cycle	Oxytocin*	Progesterone†	
Corpus luteum weight (g)	1.74 ^a	4.40 ^b	2.51 ^a	—	0.37 (9)
Concentration of progesterone (µg/g)	2.8 ^a	29.0 ^b	14.6 ^c	—	37.9 (9)
Volume of largest follicle (ml)	1.8 ^a	1.1 ^{ab}	1.4 ^{ab}	0.6 ^a	0.23 (12)
Total follicular fluid wt (g)	6.11 ^a	6.32 ^a	7.11 ^a	3.15 ^b	3.28 (12)
FSH: mean ovarian wt of test rats (mg)	68 ^a	102 ^{ab}	95 ^{ab}	113 ^b	276 (9)
LH: mean percentage ascorbic acid depletion	46.1 ^a	50.5 ^a	45.2 ^a	44.5 ^a	742 (9)

* 150 units daily from day of oestrus to Day 6 of cycle. Animals slaughtered on Day 7.

† 100 mg daily from Day 7 of the cycle to Day 41. Animals slaughtered on Day 42.

‡ Numbers in parentheses indicate degrees of freedom.

a, b, c Means in the same row bearing entirely different superscripts are significantly different from each other ($P < 0.05$).

animals had a significantly higher pituitary FSH level when compared with the Day 1 group (113 versus 68 mg, $P < 0.05$). However, when compared with the Day 7 group, the difference was not significant. The oxytocin treatment produced no significant alteration in FSH in comparison with the Day 7 group. The pituitary FSH level on the day of oestrus was found to be considerably lower than at Day 7 (68 versus 102 mg) although this difference was not statistically significant.

The LH studies revealed no significant effects due to progesterone, oxytocin or the stage of the cycle.

DISCUSSION

The results may suggest that progesterone blocks not only the release of FSH but also its synthesis, since the progesterone-treated group had a level of FSH signi-

ificantly higher than the day-of-oestrus group and a level similar to that of the Day 7 group. At the same time, the total follicular fluid weight of the progesterone-treated group was significantly less than in either of these two groups and the volume of the largest follicle showed a similar trend. It should be noted, however, that the progesterone block is partial as the ovaries still contained measurable follicles. Ray *et al.* (1961) found that a single injection of Repositol progesterone decreased the FSH concentration significantly (as well as LH) while Nellor & Cole (1957) failed to find any significant effect of one large dose of progesterone in starch suspension.

Since progesterone treatment was effective in blocking both oestrus and ovulation without altering the hypophyseal LH level, the latter hormone also seems to be affected similarly by the progesterone treatment. A curtailment of synthesis of FSH and LH and consequently of their release indicates that in this respect the cow is similar to the sheep (Bellows *et al.*, 1963) but different from the pig (Foote *et al.*, 1958; Rigor *et al.*, 1963) and rat (Van Rees, 1959a, b; Rothchild, 1962; Bellows, 1962).

Another possibility, however, is that progesterone acts on the ovary itself and interferes with the action of FSH and LH, and not on their release. Gemzell (1961) induced ovulation in the human female by administration of human gonadotrophin. Concurrent administration of progesterone with gonadotrophin was found to block this response. Hammond (1961) reported that a greater amount of gonadotrophin was required to induce ovulation during the luteal phase as compared with the follicular phase. On the other hand, there is evidence which does not support this hypothesis. Makepeace (1939) and later Pincus & Chang (1953) produced ovulations by administering exogenous gonadotrophins to rabbits under the influence of progesterone. Also ovulations can be induced during pregnancy in some species (Everett, 1961).

Oxytocin produced a significant decrease both in the weight of the corpus luteum and in the concentration of progesterone. Simmons & Hansel (1962) reported similar findings in heifers treated with oxytocin and killed 11 days post-oestrus, when some of the heifers had returned to oestrus and had ovulated again. Armstrong & Hansel (1959) were the first to report that injection of oxytocin at the beginning of the cycle inhibits the formation and function of the corpus luteum to such an extent that treated animals show precocious oestrus and ovulations.

Bioassay data on the effects of oxytocin on pituitary gonadotrophins are limited. Oxytocin treatment with 150 units produced no significant alteration in pituitary FSH or LH levels. However, Hansel, Armstrong & McEntee (1958) reported a significant hastening of ovulation by injection of oxytocin at the beginning of oestrus (50 or 60 units administered intravenously plus 50 or 100 units administered subcutaneously). This suggests the stimulation of LH release from the hypophysis; although the possibility that oxytocin acts peripherally on the follicular musculature cannot be excluded (Meyer, 1961). Martini, Pecile & Saito (1959) and Shibusawa, Saito, Fukuda, Kawai, Yawada & Tomizawa (1955) obtained increased excretion of gonadotrophin by oxytocin treatment in a number of species. The present experiment does not exclude such a possibility.

Stage effect

On the day of oestrus, the size of the corpus luteum of the preceding cycle was considerably reduced, although it still contained a detectable amount of progesterone, the function of which is unknown. Melampy, Emmerson, Rakes, Hanka & Eness (1957) reported that administration of progesterone considerably reduced the amount of oestrogen required to induce oestrus in ovariectomized heifers. Progesterone also is present in follicular fluid (Short, 1962). It is possible that progesterone in the corpus luteum plays a role along with that coming from the follicle in the induction of behavioural oestrus. In the sheep the presence of a waning corpus luteum of the preceding cycle is possibly necessary for behavioural oestrus to occur (Robinson, 1959).

No statistically significant stage effect was found either on total follicular fluid weight or on the size of the largest follicle, although in this experiment the latter was considerably larger on the day of oestrus than on Day 7 of the cycle (1.8 versus 1.1 ml). The pituitary FSH and LH levels on the day of oestrus were lower than on Day 7 but the differences were not statistically significant. Similar trends were found by Paredis (1950) and by Ray *et al.* (1961). Other species also show a similar trend (Greep, 1961).

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