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SOME PITUITARY–OVARIAN RELATIONSHIPS IN THE PERIPARTURIENT COW

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Summary. An experiment involving twenty multiparous Holstein cows was designed to study the pituitary–ovarian relationship in periparturient cows. The cows were divided into four equal groups. One group was slaughtered between 260 and 265 days of gestation; the second group was slaughtered within 18 hr following parturition; the third group was slaughtered 21 days *post partum* and the fourth group received 100 mg of progesterone daily for 20 days starting from the day of calving and was slaughtered on Day 21. There was no detectable amount of progesterone in the corpora lutea of pregnancy on the day of calving. An average of 26 μg/g progesterone was present in the corpora lutea of ante-partum cows. The corpora lutea were significantly larger in the ante-partum group than in the day-of-calving group. A significantly higher FSH level and lower LH level was found in the pituitaries of the day-of-calving group. Follicular size was significantly lower in the ante-partum and day-of-calving group as compared to both the post-partum groups. Injection of progesterone produced no significant alterations in FSH and LH levels of the pituitary glands. Neither did it depress ovarian follicular growth.

INTRODUCTION

Parturition is accompanied by a marked alteration in the pituitary–ovarian relationship. Oestrous cycles, suppressed during pregnancy, are resumed at varying lengths of time after parturition in different species. The average time after calving at which the first corpus luteum was formed in dairy cows was 19 days, while the first behavioural oestrus occurred after an average of 32 days (Menge, Mares, Tyler & Casida, 1962). The steroidal inhibition of the pituitary gland is removed at or about the time of parturition and a major inhibitor of the pituitary gland during pregnancy is considered to be progesterone. It was, therefore, presumed that by injecting this hormone, the changes which the gland undergoes immediately following parturition may be suppressed or reduced. The present experiment was conducted to study the pituitary–ovarian relationship before and after parturition and the effect of post-partum injections of progesterone upon it.

MATERIALS AND METHODS

This experiment involved four groups of five multiparous Holstein cows each. Group I (called the ante-partum group) was slaughtered between 260 and 265 days of pregnancy. The average length of gestation found in this herd is 278 days. Group II was slaughtered within 18 hr following parturition. Group III (post-partum control group) was slaughtered on Day 21 following calving. Group IV (post-partum treated group) was injected daily with 100 mg of progesterone in corn oil (subcutaneously) beginning on the day of calving and continuing through Day 20 *post partum* and killed on Day 21. Groups III and IV were milked twice daily during the 3 weeks following calving.

The methods for processing the pituitaries, ovaries and corpora lutea have been described previously (Labhsetwar, Collins, Tyler & Casida, 1964). The same modifications of the HCG augmentation test for FSH (Steelman & Pohley, 1953) and the same dosage of pituitary powder were used. However, three rats per gland were employed and the tests were performed in five replicates. With each replicate, except the first, standard FSH (NIH-FSH-S-1, ovine) at three doses (50, 100 and 200 μg) with two rats per dose was tested in the same way. Responses from the experimental glands were not translated into equivalents of standard FSH. A complete dose–response curve for the experimental glands could not be run and in a more recent study (J. W. Riesen, personal communication, 1963) ovine and bovine unfractionated extracts gave different shaped dose–response curves using the Steelman & Pohley test method.

The pituitary LH level was tested by the ovarian ascorbic acid depletion method of Parlow (1958). The lyophilized powder in the required quantity was suspended in saline and allowed to stand refrigerated overnight. On the following day the suspension was ground in a homogenizer and centrifuged at 480 *g*. The supernatant solution was then diluted to obtain three doses (1·20, 0·40 and 0·13 mg) of dry powder per ml. All the injections were made with a constant volume of 1 ml saline via a tail vein. Two rats per dose were employed. Four hours ±10 min after the injection both ovaries were obtained and processed together to determine ascorbic acid concentration. With each replicate a standard LH (NIH-LH-S-3, ovine) at three dose levels (3·30, 1·10 and 0·37 μg) with two rats at each dose was also run. The ascorbic acid (AA) was adjusted for ovarian weight by covariance (Sakiz & Guillemin, 1963). The mean concentration of AA in μg/100 mg of ovaries of the three doses was used as an index of LH activity.

The progesterone determinations were carried out by the method of Stormshak, Inskeep, Lynn, Pope & Casida (1963).

Statistical analysis of the data was performed as described by Labhsetwar *et al.* (1964).

RESULTS

Corpus luteum and progesterone concentration

None of the cows treated with progesterone showed a detectable oestrus during the experimental period; no new corpora lutea were present in these animals at the time of slaughter. In the post-partum control group two cows showed ovula-

tions without having expressed behavioural oestrus, one showed oestrus 2 days prior to slaughter and the remaining two had no corpora lutea in their ovaries. Corpora lutea of pregnancy were still present in all the animals in the day-of-calving group, although they were significantly smaller in comparison to those in the ante-partum group (5·52 versus 6·73 g, $P<0·05$). The corpora lutea in the former group contained no detectable progesterone while an average of 26 μg/g of progesterone was found in the latter group.

Volume of the largest follicle and total follicular fluid weight

No grossly measurable follicles were found in the prepartum and day-of-calving groups. Treatment with progesterone was accompanied by slightly but

TABLE 1

INFLUENCE OF STAGE OF GESTATION, PARTURITION AND PROGESTERONE ON VARIOUS PARAMETERS

Parameter	*Group*				*Error mean square*‡
	I. *Ante-partum* (260 *to* 265 *days p.c.*)	II. *Day of calving*	III. 21 *days post partum*	IV. 21 *days post partum* + *progesterone**	
Corpus luteum wt (g)	6·73[a]	5·52[b]	—	—	0·50 (7)
Concentration of progesterone (μg/g)	26	Undetected	—	—	—
Volume of largest follicle (ml)	—	—	2·3[a]	1·6[a]	1·93 (8)
Total follicular fluid wt (g)	2·38[a]	1·48[a]	4·66[b]	5·63[b]	2·05 (16)
Pituitary dry wt (mg)	431[ab]	320[a]	414[ab]	503[b]	7124 (16)
FSH: mean ovarian wt of test rats (mg)	163[ac]	199[a]	98[bc]	80[b]	5050 (12)
LH: AA in ovarian tissue (μg/100 mg)†	66·0[ab]	74·1[a]	53·8[b]	54·5[b]	77 (12)

* 100 mg daily from the day of calving to Day 20 *post partum*.
† Concentration of ascorbic acid adjusted for ovarian weight.
‡ Numbers in parentheses indicate degrees of freedom.
[a, b, c] Means on the same line bearing entirely different superscripts are significantly different from each other ($P<0·05$).

not significantly smaller follicles as compared to the post-partum control group (1·58 versus 2·26 ml, volume of largest follicle). The ante-partum and day-of-calving groups had significantly smaller follicular fluid weights as compared to the post-partum groups (Table 1). Again the treatment with progesterone was found to have no significant effect on the total follicular fluid weight when compared to the post-partum control group. Follicular development had occurred, however, during the period of treatment after parturition.

Pituitary studies

The day-of-calving group had the highest pituitary FSH level (199 mg ovarian weight of test rats, Table 1) while the post-partum group treated with progesterone

had the lowest (80 mg, Table 1). The pituitary level found in the ante-partum group (163 mg) was not significantly different from the day-of-calving group. The latter had a significantly higher level than either of the two post-partum groups. The prepartum group was significantly higher than the post-partum group treated with progesterone. The difference between the ante-partum and the control post-partum group was not significant, although there was a strong tendency towards a higher level in the former group (163 versus 98 mg). Treatment with progesterone produced no significant alteration in pituitary FSH (80 versus 98 mg for the progesterone-treated post-partum group versus control post-partum group).

Significant group differences were also detected in pituitary LH in terms of AA/100 mg of ovarian tissue. The slopes of the average dose–response for the experimental glands and that for standard NIH-LH (ovine) were significantly different (regressions, 11·2 versus 19·1, $P<0·05$). Due to this difference of regressions, the standard LH curve could not be used to translate the AA concentration into equivalents of LH. This precluded the estimation of total LH.

The day-of-calving group had the lowest LH level, i.e. the highest AA concentration per 100 mg of ovarian tissue in the test rats (Table 1). This was significantly lower than both the post-partum groups but not the ante-partum group. Treatment with progesterone produced no significant alteration in LH level in comparison to the control post-partum group (54·5 versus 53·8 μg of AA/100 mg of ovarian tissue). There was a tendency towards a lower level in the ante-partum group in comparison to both the post-partum groups, although it was not statistically significant.

The day-of-calving group which had the highest pituitary FSH level had the smallest dry pituitary weight (320 mg) and the post-partum treated group which had the lowest FSH level had the largest dry pituitary weight (503 mg). No significant correlation was evident within the groups between the dry weight of glands and level of FSH. Also, Nalbandov & Casida (1940) did not find a significant correlation between the unit gonadotrophic potency and weight of the gland. As stated before, total FSH and LH content could not be calculated due to differences in curves for standards and unknowns.

DISCUSSION

Significantly smaller corpora lutea in the day-of-calving group in comparison to the ante-partum group indicate that some degeneration is undergone during the last days of gestation. The absence of progesterone in a detectable quantity on the day of calving in contrast to its presence in relatively higher concentration 10 to 15 days before (26 μg/g) supports this conclusion further. Short (1960) found a decrease in the blood level of progesterone before calving.

There were no grossly measurable follicles in the ante-partum and day-of-calving groups and the total follicular fluid weight in these two groups was also significantly lower than in either of the post-partum groups. A significant decrease in follicular size throughout pregnancy in cows was reported by Nalbandov & Casida (1940). Treatment with progesterone produced no significant depression either in the size of the largest follicle or the total follicular

fluid weight. This is in contrast to non-pregnant heifers (Labhsetwar *et al.*, 1964) although in the latter case injections were made for 35 days as against 20 days in the present study. Besides the difference in injection interval there may be differences in the physiological conditions of post-partum cows as compared to non-pregnant heifers, particularly due to the existence of a 'rebound phenomenon' in the post-partum group (Lakshman & Nelson, 1963).

When the follicular growth in the ovaries is correlated with the gonadotrophic level in the gland further important changes due to parturition become apparent. A high pituitary FSH level was present on the day of calving and a significant reduction occurred within 21 days following parturition. However, the ovarian picture at these two stages is altogether different. In spite of a greater pituitary level on the day of calving, follicular size in this group is significantly lower than in the 21-days post-partum group. This would suggest that the rate of secretion of FSH in this group is lower as compared to the post-partum group. The lack of follicular growth in the day-of-calving group is probably not due to an ovarian insensitivity to gonadotrophins as ovaries at this stage are capable of responding to exogenous gonadotrophins (Casida, Meyer, McShan & Wisnicky, 1943). Therefore, the higher FSH level on the day of calving seems to be due to the rate of FSH synthesis having been higher for a period of time than the rate of its release from the gland.

Nalbandov & Casida (1940) found a steady and significant decrease in the gonadotrophic potency throughout pregnancy which is in contrast to the upward trend in pituitary FSH from the prepartum to the day-of-calving group in this study. These two observations can be harmonized if one postulates a gradual release of the pituitary gland from steroidal inhibition in the terminal stages of gestation. Absence of progesterone on the day of calving supports this hypothesis in part. Failure of Nalbandov & Casida (1940) to find this increase of FSH at the terminal stages of gestation may have been due to their material having been obtained from the slaughter house and the likely absence of truly terminal stages. Their assays also did not differentiate between the individual gonadotrophins.

Following parturition the anterior pituitary undergoes significant alterations for then the gland contains a significantly lower level of FSH and a significantly higher level of LH. Thus, reversal in the ratio of FSH/LH occurred; but this was associated with follicular growth in the ovaries. It appears that during this interval the rate of release of FSH may for a time have exceeded the rate of synthesis and therefore the temporary increase of FSH during the terminal stages of pregnancy disappears following parturition. The kinetics of LH production and release cannot be deduced since follicular growth is not a good index of LH secretion. The occurrence of ovulation in three of five animals seems to be consistent with the higher level of LH in the post-partum group than in the day-of-calving group.

Injection of progesterone failed to maintain the pituitary gland and ovaries in the condition which existed during pregnancy. Large quantities of oestrogen are excreted in the pregnant animal (Nelson & Smith, 1963). Therefore, this failure may have been due to the lack of oestrogen to sensitize the post-partum animal to the effects of progesterone (McCann, 1962).

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