THE USE OF HORMONES IN INDUCING OVULATION AND SUBSEQUENT FERTILITY IN DOMESTIC ANIMALS

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Here to fore good results have been obtained clinically by the use of follicular stimulating hormone, formerly known as prolan—Aschheim-Zondek, to obtain reproduction in barren animals such as bitches as well as in women. Methods of producing ovulation in the mature domestic animals have been studied, and also with the incidence of ovulation in animals treated in different reproductive stages, and the potential fertility of the eggs that have been ovulated by artificial means. The followings are current summaries of some pertinent experimental data.

Early work with the juvenile rabbit indicated that pituitary follicle stimulating extracts[2] could be administered subcutaneously for a few days to develop a number of follicles in the ovary, and that a luteinizing extract of the pituitary gland could then be administered intravenously to bring about ovulation of these follicles. The work was next extended to immature females of various other species. The same method of treatment would bring about ovulation in the pigs of four months of age, in the lambs of three to five months of age, and in the calves of two to four weeks of age.

Ovulation of a larger number of eggs than was normal for the adult of the various species was the rule in these experiments. It would seem there was a tendency for the follicle stimulating extract to produce approximately equal growth in a large number of follicles rather than to select a few follicles and produce a large amount of growth in them. This condition appeared in all the species, whether they were litter bearing animals er whether their inherent ovulation rate was normally one or two.

Exploratory studies were next made using follicle-stimulating extract[4] and luteinzing or unfractionated extracts in mature cows and in cows during the post-partum interval. Many attempts were made to regulate the number of follicles that would ovulate by varying the dosage of follicle stimulating extract used in the pre-treatment before the ovulation stimulus was given. Relatively little success was obtained in this regard. There appeared to be some correlation between dosage and number of ovulations, but for any one particular animal

the degree of predictability was exceedingly low.

Larger numbers and more uniform animals were available usually for studies with sheep than with cattle. A comparison was made of the ovulation rates of sheep treated in the luteal phase and in the follicular phase of the estrual cycle. Although all ewes ovulated, the number of corpora lutea and the number of eggs that were recovered by flushing of the oviducts appeared to be greater considerably in those sheep treated in the follicular phase. The potentialiy of super-ovulation following a standard treatment with follicle stimulating extract appeared, then, to vary with the stage of the estrual cycle at the time of treatment.

The point of view which had been developed in the course of these experiments was that the action of the treatment with follicle–stimulating extract was to increase the number of follicles that were susceptible to the ovulation stimulus. A few medium to large size follicles are present in the ovaries of untreated animals at most stages of estrual cycle. A comparison was next made of the ovulation rates in ewes representing two phases of the estrual cycle which received the intravenous injection only. Ovulation occurred in all the animals at each stage, and there was no evidence of a stage difference in the number of corpora lutea formed. In general, the number of ovulations was normal for the species.

Simultaneous studies were under way on the effect of stage of the reproductive cycle upon ovulation rate in the rabbit. A comparison was made between the ovulation rate in the rabbits that were in estrus and in those that were ten days pseudopregnant. Here again when the pre-treatment with follicle stimulating extract was used, the number of corpora lutea and the number of recovered eggs were greater in the estrual or follicular phase rabbits than in pseudopregnant or or luteal phase ones. When the follicle stimulating treatment was omitted, the number of ovulations were comparable in the two groups and they were characteristic for the species. Further studies[8] were made by including pseudopregnancy in Dutch and Himalayan rabbits by intravenous injection of 100 I. U. of chorionic gonadotrophin. The ovulation of a second set of follicles was achieved 3–16 days later by the similar admistration of 50 I. U. of the same substance and insemination was performed simultaneously. Fertilization, judged by the presence of segmenting ova in the tubal washings, occurred only in the early (third day) and late (fourteenth to sixteenth day) pseudopregnancy. Failure to achieve fertilization during the greater

part (fourth to thirteenth day) of pregnancy is attributed to the inability of an adequate number of sperms to ascend the fallopian tubes due to the conditions prevailing in the uterus. It was observed by inducing epididymal sperms through the infundibulum into the fallopian tubes, that only a small proportion of the eggs shed at induced ovulation during pseudopregnancy were fertilizable.

Previously fertilized eggs (two cells stage) introduced into the fallopian tubes of pseudopregnant and estrous rabbits continued to segment and a large proportion can be recovered later from the tubes as blastocysts; fertilized eggs implanted into rabbits in these two conditions failed to pass from the tubes into the uterus. The estrogen-progesterone balance is important in relation to the function of the fallopian tube in the transportation of fertilized eggs.

A report by Hammond et al.[3] indicated that ovulation could be brought about in the ewe by the admistration of estrogen. Subsequent reports by various other workers indicated that the response was at least not a predictable one. A comparion was made of the incidence of ovulation in the ewe when treated with diethyl stilbestrol diproprionate at different stages of the estrual cycle. Ovulation was obtained most consistently when the hormone was administered at the fourth day of the estrual cycle and was obtained rarely at other stages. Again the number of ovulation per ewe was characteristic for the species.

It is clear that the production of ovulation is but one step in the aritificial induction of fertility in the female. The question next arises as to whether the eggs are fertilizable. In the initial work on superovulation in cattle, attempts were made to induce superfecundity in the cow when treated in the luteal phase and in the follicular phase of the estrual cycle. No palpable pregnancies were obtained in cow treated during the luteal phase but a normal percentage of pregnancies occurred in those treated during the follicular phase. The larger than normal number of fetuses present in some of the latter animals indicated that eggs from artificially matured and ovulated follicles must have been fertilizable in the follicular stage.

Fertility of Experimentally Ovulated Cow Eggs

Reproductive stage	*Number cows ovulating*	*Number palpable pregnancies*
Luteal phase	4	0
Follicular phase	6	4*

*3 cows at 33 to 50 days; 4,4 and 7 embryos present.

The work was next transferred to sheep[6]. Here again the potential fertility of the eggs resulting from superovulation appeared to be determined by the stage of estrual cycle at which treatment was initiated. No fertilized eggs were found in the luteal phase ewes, and there was evidence that the degree of fertility obtained in the follicular phase ewes depended on the particular batch of follicle stimulating extract that was used in the pretreatment of the animals.

It was evident that data on three different species, cattle, sheep, and rabbit, all could point in the same direction—that there is some action of the corpus luteum itself that has an inhibitory effect on fertility of the artificially ovulated eggs. Further studies were next made to determine whether the hormone of the corpus luteum, progesterone, when administered to an animal in the follicular stage for a time before experimental ovulation, would inhibit the fertilizability of the eggs. Two mg. of progesterone daily for ten days before experimental ovulation reduced the incidence of fertilization in the originally estrual rabbit to a very low value on the average, and in most animals no fertility was observed. There has been no opportunity as yet to test for this inhibitory action of progesterone in cattle and in sheep. From the data on the rabbit, however, it is suggested that the low fertility in the animals treated during the luteal phase of the reproductive cycle is due to some physiological action of progesterone.

Fertility of Experimentally Ovulated rabbit Eggs

Stage when treated	Number of females	Total number of eggs	Per cent eggs cleaving equally
Estrual	6	46	94
3 days pseudopregnant	6	25	80
5 days pseudopregnant	6	50	22
10 days pseudopregnant	5	32	0
13 days pseudopregnant	5	42	6

Effects of Progesterone on Fertility of Experimentally Ovulated Eggs of Rabbits Initially in Estrus

Treatment before ovulation	Number of famales	Total number of eggs	Per cent eggs cleaving eqally
No progesterone	11	90	96
Two mg. progesterone daily for 10 days	15	116	5

The above data also explain failure to pass from fallopian tubes in the uterus of implanted fertilized eggs in pseudo-pregnant and estrous rabbits as stated in page 4.

Equine gonadotrophin was applied successfully in sows[11]. Forty two sows were injected with approximately 1000 I. U. equine gonadadotrophin during lactation to induce estrus. Sows which were injected in early lactation (1 to 38 days) did not manifest estrus regularly, but 26 of 27 sows injected between the thirty-ninth and sixty-eighth days of lactation came into heat three to seven days following treatment. All of these animals were bred. Twenty of twenty three, on which information was available, became pregnant during the induced estrus.

These experiments indicate that the time between successive farrowings can be shortened by two to four weeks by the use of equine gonadotrophin on or after the fortieth day of lactation. A distinct advantage is that the farrowing season can be concentrated within a short period by allowing the early farrowing sows to breed normally at the end of lactation and inducing estrus in the late farrowing sows during lactation. Furthermore, it becomes possible to allow the pigs to suckle longer, if desirable, and still obtain two litters per year.

In this connection it may be well to state a few words regarding the use of hormones in treating sterility in animals. Where adequate controlled experiments in treating sterility in cattle have been set up hormonic treatment has failed to demonstrate efficiency. It appears that we know too little about the exact rôle played by the hormones and about the dosages that should be employed. Much more back ground work needs to be done and many more critical field trials.[9]

Endocrinology in its application to farm practices has not yet attained that pitch of disseminated usefullness now assumed by the sciences of nutrition or even chemotherapy ; but its possibilities are equally great and it is important that they should not be too severely prejudged at this stage. The hormonal treatment given in the field of animal husbandry at the present time – and too for some time to come–remains experimental in nature, Their use, regarding hormones, to date has met with only partial success, because of lack of knowledge to the function of the endocrine secretions.[10]

In his lecture at Michigan State College on February 22,50 L.E. Casida explained the cause of cystic ovary in bovine as due to persisting tissue of corpus luteum. This produces progesterone which acts on the pituitary inhibiting luteinizing hormone with cystic ovary as the result. He claimed good result in

the treatment wit a single intravencus injection of 10,000 I.U. chorionic gonadotrophin obtained from pregnant woman urine.

Interpretation

In interpreting the results of most of the above experiments, one is impressed first with the difficulties that will be encountered in inducing fertility in a whole herd or flock of breeding females at one time. Al though ovulation may be induced with considerable certainty at various times in the estrual cycle, the potential fertility of the eggs will still be determined by the animal's basic physiological stage. So far the artificial manipulation of changes in the ovary is still largely superficial and does not take command of the pattern of fertile periods which is intrinsic to the animal.

A question which is raised by the findings has to do with the phenomenon of superfoetation. If ovulation should occur during pregnancy and while abundant supply of progesterone is operative, the chances for the aberrantly ovulated eggs being fertilizable would appear to be small. If superfoetation does not occur, is there a transitory period when the progesterone level falls very low while the new follicle matures and ovulates and fertilization of the egg ensues? Can the pregnancy survive a short progesterone drought at certain stages until a new corpus luteum is formed? The physiological mechanism whereby progesterone interferes with fertilization is of commanding interest. Its effect might be directly or indirectly on the egg itself, rendering it unable to respond to the fertilization stimulus. On the other hand, some eggs may be capable of fertilization, but the environment in the genital tract of the female, and through which the sperms must travel and effect fertilization, may be such that the potential fertility of the spermatozoa is lost. An attempt was made to demonstrate a possible difference in the rate of travel of the sperm cells through the genital tracts of experimental and of normal females. No clear cut difference was demonstrable. Even though the sperm cells may traverse the tract and reach the eggs, it seems possible that they may have lost their potential fertility by some action of the genital environment upon them. In further studies, sperm cells were subjected to media of finely hashed endometrium from pseudopregnant rabbits for variable times before they were used in the insemination of normal animals. No impairment of their ability to fertilize normal eggs could ever be demonstrated.

If the hormone effects the egg itself so that it is incapable of being fertilized, it might be so before the time of ovula-

tion. One line of regression on this problem takes as its point of departure the apparent fact that a crop of follicles is matured in preparation for estrus. This crop contains many more follicles than ovulated. Those that are not ovulated eventually degenerate, but they may continue their development for some time after the heat period. In the sheep the average size of follicles appears to be larger at some time near mid-cycle than at any other stage. Can it be that the follicles which respond to the artificial growth and ovulation stimuli during the luteal phase of the cycle are actually culls from the last crop that developed? The last natural ovulation removed some of the follicles at a time that the eggs were fertilizable, but the remaining ones which for some time can be removed from the ovary by an artificial ovulation stimulus lose their potential fertility either as a function of age or as a result of the action of progesterone from the new corpora lutea. The demonstration that corpora lutea may inhibit the potential fertility of otherwise fertilizable eggs suggests that the corpora lutea may bring about this action in the normal cycle.

Summary

Intravenous injection of an unfractionated extract or of a luteinizing extract of sheep pituitary glands was an effective means of inducing ovulation in cattle, sheep, and rabbits. The number of ovulations induced by this treatment was characteristic for the species.

The time between successive farrowings and the farrowing seasons in a herd of swine can be shortened and concentrated within a short period respectively by the use of equine gonadotrophin.

Treatment with a follicle stimulating extract subcutaneously for a few days before the ovulation stimulus was given resulted in superovulation, and species differences in numbers of ovulations were not clearly discernible. The number of ovulations tend to be less when treatment was given during the luteal phase of the reproductive cycle than when given during the follicular phase.

Ovulation in the ewe was induced rather consistently when diethyl stilbestrol diproprionate was administered on the fourth day of the estrual cycle, but rarely when it was administered on other selected days of the estrual cycle.

Potential fertility of the ova that were ovulated by use of pituitary gonadotrophins was affectedly marked by the stage or phase of the reproductive cycle at time the of treatment. Ovulations that were increased during the luteal phase yielded eggs of low fertility; those during the follicular phase were of normal

fertility. Evidence for this condition was obtained in cattle, sheep, and rabbits.

Administration of progesterone to estrual rabbits for ten days before the ovulation treatment was given resulted in a normal ovulation rate, but the eggs were of very low fertility.

Good result has been claimed by the use of chorionic gonadotrophin in the treatment of cystic ovary in cattle.

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