

## EFFECTS OF PROGESTERONE ON FERTILIZATION AND EGG TRANSPORT IN THE PIG

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Polyspermy can be produced experimentally in pigs by delayed mating, which results in the fertilization of aged eggs (Pitkjanen, 1955; Thibault, 1959; Hancock, 1959; Dziuk & Polge, 1962; Hunter, 1967). A high incidence of polyspermy has also been observed in pigs following induced ovulation and insemination during the luteal phase of the oestrous cycle (Hunter, 1966). These observations suggest that the endogenous level of progesterone at the time of fertilization might affect the block to polyspermy in pig eggs, since, under both conditions cited, developing or fully functional corpora lutea would be present in the ovaries at the time of sperm penetration. The present study was conducted to determine the frequency of polyspermy in pigs injected with progesterone at various intervals before ovulation and fertilization.

The experimental animals were thirty-six mature, crossbred, Large White × Essex, gilts. The time of ovulation was controlled by the injection of 500 i.u. human chorionic gonadotrophin (HCG) given intramuscularly during late pro-oestrus or at the onset of oestrus. Ovulation would then occur 40 to 42 hr after HCG (Dziuk & Baker, 1962; Dziuk, Polge & Rowson, 1964). A single subcutaneous injection of 100 mg progesterone in 6.6 ml arachis oil was given to groups of six gilts at 12, 18, 24 or 36 hr before ovulation. A control group was injected with oil alone 36 hr before ovulation. All animals were inseminated with 80 to 100 ml of fresh undiluted semen 12 to 24 hr before ovulation. The above groups were killed at approximately 8 hr after ovulation for egg recovery, but in an additional group injected with progesterone 18 hr before ovulation, egg recovery was delayed until 23 hr after ovulation. At autopsy, the reproductive tracts were removed and the oviducts and uterine horns were flushed separately with physiological saline. The eggs which were recovered were prepared as whole mounts, fixed and cleared in 25% acetic-alcohol, stained with 1% aceto-orcein and examined by phase-contrast microscopy.

The results are shown in Table 1. Fertilized eggs, some of which were polyspermic, were recovered from all groups, but the percentage of eggs fertilized was reduced and the incidence of polyspermy was greatly increased in animals injected with progesterone 24 or 36 hr before ovulation. In these two groups, seven out of the eight gilts from which fertilized eggs were obtained

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TABLE I  
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Hours from progesterone injection to ovulation	Hours from ovulation to egg recovery	No. animals	No. ovulations	No. eggs recovered	% eggs fertilized	% fertilized eggs polyspermic	% eggs in uterus
Control	8	6	83	78	94	8	0
12	8	6	79	77	71	18	0
18	8	6	92	90	93	13	0
24	8	6	89	86	52	40	0
36	8	6	84	69	64	36	77
18	23	6	72	49	76	—*	61

\* Eggs recovered at this stage could not be classified accurately for polyspermy.

had one or more eggs penetrated by several sperm and 36 to 40% of all the fertilized eggs were polyspermic. The incidence of polyspermy in eggs recovered from control animals, which received arachis oil alone, was higher than that previously noted in gilts injected with HCG during pro-oestrus and inseminated before ovulation (Hunter, 1967). However, the frequency of polyspermy in gilts injected with progesterone 12 or 18 hr before ovulation was similar to that observed in gilts following delayed mating (Thibault, 1959) or following insemination 10 to 14 hr after ovulation (Hunter, 1967). Hancock (1959) reported that the incidence of trinuclear eggs in pigs mated 40 to 48 hr after the onset of oestrus was 29%, which is a figure more closely approaching that found in this study when pigs were injected with progesterone 24 or 36 hr before ovulation.

The number of extra spermatozoa found within the polyspermic eggs varied from one to over thirty. In most eggs the sperm heads had developed into male pronuclei, which differs from the situation observed in immature eggs where little or no change occurs in the sperm heads following penetration of the vitellus (Polge & Dziuk, 1965). The presence within the eggs of sperm tails, which were generally split at the mid-piece and often associated with pronuclei, confirmed the identification of extra pronuclei as having been derived from sperm. There was no evidence of digyny as has been reported following delayed mating in the sow (Thibault, 1959).

An accelerated rate of egg transport through the oviducts was also caused by injecting progesterone before ovulation. Seventy-seven per cent of the eggs recovered from gilts which were injected with progesterone 36 hr before ovulation and killed 8 hr after ovulation were found in the uterine horns (Table 1). These eggs were in the pronucleate stage of development and some of them were still surrounded by cumulus cells. Similarly, in the additional group of six gilts injected with progesterone 18 hr before ovulation, but in which egg recovery was delayed until 23 hr after ovulation, 61% of the eggs had reached the uterus. The recovery rate of eggs in both of these groups was low, suggesting that some eggs might even have been expelled from the uterus into the vagina. In these animals, the time from progesterone injection to egg recovery was 41 to 44 hr. By contrast, when the time from progesterone injection to egg recovery was only 20 to 32 hr, as in animals injected with progesterone 12, 18 or 24 hr before ovulation and killed 8 hr after ovulation, all eggs were still found within the oviducts. Chang (1966) also found that the administration of progesterone before ovulation speeds the transport of eggs through the reproductive tract in rabbits.

These studies emphasize the fact that fertilization, development and transport of eggs in mammals can be influenced by the levels of ovarian hormones. The reason why increased progesterone level at the time of fertilization in the pig appears to interfere with the normal block to polyspermy of the egg is not evident, but it could be due either to a direct effect of progesterone on the egg or to an alteration in the environment of the oviduct. The results may provide an explanation as to why delayed mating frequently results in an increased incidence of polyspermy and, in addition, suggest a causal relationship between progesterone level following ovulation and rate of egg transport in the pig.

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