RELEASE OF PROGESTERONE FROM SILICONE RUBBER IMPLANTS *IN VITRO*, AND THE EFFECTS OF THE IMPLANTS ON PLASMA PROGESTERONE LEVELS IN SHEEP

N. F. CUNNINGHAM, N. SABA AND P. G. MILLAR

*Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory,
New Haw, Weybridge, Surrey KT15 3NB*

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The effects of silicone rubber progesterone implants on plasma progesterone levels in anoestrous ewes have been reported by Symons, Cunningham, Saba & Millar (1974). Plasma progesterone rose from a mean preimplantation value of 0.18 ng/ml to 0.58 ng/ml 24 hr after implantation. The mean level then declined and by 10 days was not significantly different from the preimplantation value. Such short-lived elevations of plasma progesterone would probably not be effective in suppressing ovulation in cyclic sheep. For the synchronization of oestrus in cyclic ewes by inhibition of ovulation, or the induction of oestrus in anoestrous ewes, it would be desirable to maintain plasma progesterone levels above 1 ng/ml for 10 to 14 days. Thus, we have studied the release of progesterone from various silicone rubber implants *in vitro*, and have investigated the effect of these implants *in vivo* on plasma progesterone levels in ewes and wethers.

In the first trial, implants were prepared as previously described (Symons *et al.*, 1974) from Silastic tubing (Dow-Corning International Ltd) of different diameters and wall thickness. Each implant contained 100 mg solid progesterone (Koch-Light Laboratories Ltd). For the experiments *in vitro*, the implants were incubated at 38°C with gentle shaking in 100 ml of 0.9% (w/v) NaCl. Aliquots (5 ml) of the incubation medium were removed and replaced with fresh medium at approximately hourly intervals for a period of 6 hr, and then at 24 hr. The samples of medium were diluted with an equal volume of AR ethanol, and their extinction values were measured at 245 nm. Progesterone concentration was determined by comparison with standard solutions of progesterone (2 to 20 µg/ml) in ethanol:0.9% NaCl (1:1 v/v).

Progesterone concentrations in the incubation media increased progressively and the concentrations after 6 hr and 24 hr are shown in Table 1.

The thin-walled implants (Nos 1 to 5) appeared to release progesterone into the medium at a faster rate than the thick-walled implant (No. 6), and experiments were carried out *in vivo* to determine whether these thin-walled implants would produce higher plasma progesterone levels than the thick-walled implant tested earlier (Symons *et al.*, 1974). Implants Nos 2 to 5 were autoclaved (20 min at 5 lb/in²) and were inserted subcutaneously in four ewes during

September. Plasma progesterone levels were determined in jugular vein samples by the radioimmunoassay method previously described (Symons, 1973). Preimplantation progesterone levels were high (0.4 to 0.9 ng/ml plasma, mean 0.74), suggesting that the ewes were coming into season. However, 20 hr after insertion of the implants, plasma progesterone levels were elevated in all four ewes (mean 1.40 ng/ml, range 0.95 to 2.3). After 2 days, the mean plasma progesterone level had decreased to 1.04 ng/ml and after 3 days it had returned to preimplantation levels (mean 0.72 ng/ml). The thin-walled implants thus produced only short-lived elevations of plasma progesterone levels in sheep.

Table 1. Progesterone concentration in saline during incubation *in vitro* of silicone rubber progesterone implants

Implant No.	Internal diam. (mm)	Wall thickness (mm)	Length (cm)	Progesterone concentration ($\mu\text{g/ml}$)	
				After 6 hr	After 24 hr
1	1.47	0.25	10	4.8	9.3
2	1.57	0.42	10	4.2	9.4
3	1.57	0.42	7.5	3.8	8.2
4	1.98	0.60	10	4.8	9.4
5	1.98	0.60	6	4.1	9.1
6	3.18	1.59	7.5	3.2	5.9

Each implant contained initially 100 mg progesterone, and was incubated in 100 ml of 0.9% NaCl at 38°C.

Table 2. Progesterone concentration in saline during incubation *in vitro* of silicone rubber progesterone implants

Implant No.	Progesterone content (mg)	Progesterone concentration ($\mu\text{g/ml}$)	
		After 6 hr	After 24 hr
8	22.5*	3.4	5.9
9	22.5	5.1	9.4
10	45	4.6	9.2
11	90	4.7	9.2
12	300	5.2	9.8

Each implant was incubated in 160 ml of 0.9% NaCl at 38°C.

* Dissolved in 0.9 ml arachis oil.

In order to increase the surface area available for the release of progesterone from the implants, triple tubing implants were prepared by sealing together longitudinally three 10-cm lengths of Silastic tubing (wall thickness, 0.60 mm). Implant No. 8 contained progesterone dissolved in arachis oil, and implants Nos 9 to 12 contained 22.5 to 300 mg solid progesterone. The results of incubation of these implants *in vitro* are shown in Table 2.

The rate of release of progesterone from implant No. 8 (progesterone in oil)

was slower than that from implants containing solid progesterone. For implants Nos 9 to 12, the concentration of progesterone in the medium after incubation for 6 hr was little different from that in the first experiment with single tubing (see Table 1, implant No. 4). The total amount of progesterone released, however, was higher in the second experiment, since the volume of the incubation medium was 160 ml compared with 100 ml in the first experiment. The release of progesterone from implants Nos 9 to 12 during incubation for 24 hr was not related to the amount of progesterone originally present in the implants (22.5 to 300 mg).

When the triple tubing implants were tested in sheep, plasma progesterone levels were again elevated for only 2 days.

A third trial was carried out with solid silicone rubber progesterone implants prepared according to the method of Mauer, Revenal, Johnson, Moyer, Hirata & White (1972). Progesterone was incorporated into Silastic medical grade elastomer, which was then polymerized by the addition of catalyst M.

TEXT-FIG. 1. Plasma progesterone concentrations in wethers with subcutaneous silicone rubber implants containing 9.09% (●) or 13.04% (○) progesterone. Each point represents the mean value ($\pm$ S.E.M., vertical bars) for five animals.

The mixture was forced into moulds 31 cm long and 0.5 cm in diameter and, when set, the rods were cut into 10-cm lengths. Implants were prepared containing 9.09% (w/w) and 13.04% (w/w) progesterone in Silastic, containing a total of 205 and 293 mg progesterone, respectively.

The implants were not tested *in vitro*, but were inserted subcutaneously in ten wethers aged about 8 months. Wethers were chosen for this experiment, since they were expected to have consistently low basal levels of plasma progesterone. The implants were left in position for 43 days. The mean plasma progesterone levels for the wethers during this experiment are shown in Text-fig. 1. The levels rose rapidly after insertion of the implants, and had reached near-maximum values within 5 hr. Peak plasma progesterone levels were observed 1 to 2 days after insertion of the implants, and up to this time the 9% implants tended to produce slightly higher progesterone levels than the 13% implants. The levels then decreased gradually, the fall being more rapid with the 9% implants.

Plasma progesterone levels were still greater than 1 ng/ml with the 13% implants 17 days after implantation. By 43 days after implantation, the levels had fallen to 0.52 ng/ml (9%) and 0.59 ng/ml (13%), values which were significantly higher ($P < 0.01$) than the preimplantation levels (0.22 and 0.26 ng/ml, respectively).

Removal of the implants after 43 days resulted in a significant fall in plasma progesterone concentration at 24 hr ($P < 0.05$) to levels (0.25 and 0.31 ng/ml) which were not significantly different from the preimplantation values. After removal from the wethers the implants were dried and weighed. In the case of the 9% progesterone implants, there was a mean loss of weight of 162.4 mg (range 159 to 168 mg). The 13% progesterone implants showed a mean loss of 189 mg (range 187 to 192 mg). If these decreases were due solely to loss of progesterone, then they represented losses of 79.2% (9% implants) and 64.5% (13% implants) of the progesterone originally present. Over the 43-day period of implantation, they would correspond to average losses of 3.8 and 4.4 mg/day respectively. These figures are consistent with the weight decreases found by Mauer *et al.* (1972) using 5% and 10% progesterone implants in ewes. Over a 14-day period of implantation, their implants lost 3.9 and 6.1 mg/day, respectively. Evidence that the weight decreases of the implants did in fact represent loss of progesterone was obtained by inserting similar silicone rubber implants containing no progesterone into six sheep. These implants showed a mean loss of weight of only 2 mg over an 11-day period.

We conclude that, for inducing and maintaining elevated plasma levels of progesterone in sheep, the solid silicone rubber progesterone implants are superior to implants made from silicone rubber tubing containing solid progesterone. Solid silicone rubber implants containing 10% (w/w) of progesterone are available commercially (Sil-Estrus, Abbot Laboratories Ltd) for the synchronization of oestrus in sheep but, to our knowledge, no data have been published on the effects of these implants on plasma progesterone levels in sheep.

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