Scientific Protocols Used When Evaluating The New Absolute AMG Series Catheter Compared To Traditional Foam Catheters

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Aims

-To compare the sperm distribution in the Utero-tubal junction (UTJ) and the oviduct (comparing AI and IUI)
-To compare fertilization rate (comparing between AI and IUI)
-To compare pregnancy rate (comparing between AI and IUI)
-To compare farrowing rate (comparing between AI and IUI)
-To compare litter size (comparing between AI and IUI)
-To compare the dose of insemination (1.5 vs 3.0 x 10^9 spermatozoa)

Experimental design

Altogether 60 sows were divided into 4 groups (15 sows in each group). They were purchased from a commercial farm and kept at Mahidol University.

Group-A (15 sows): inseminated by using **a foam tip** with a dose of **1.5 x 10^9** spermatozoa.

Gr.-A1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)
Gr.-A2 (5 sows): slaughter to see the fertilization rate (3 days after AI)
Gr.-A3 (5 sows): to see the pregnancy rate, farrowing rate and litter size

Group-B (15 sows): inseminated by using a **foam tip** with a dose of **3.0 x 10^9** spermatozoa.

Gr.-B1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)

Gr.-B2 (5 sows): slaughter to see the fertilization rate (3 days after AI)

Gr.-B3 (5 sows): to see the pregnancy rate, farrowing rate and litter size

Group-C (15 sows): inseminated by using a **new catheter (the Absolute AMG Series)** with a dose of **1.5 x 10^9** spermatozoa.

Gr.-C1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)

Gr.-C2 (5 sows): slaughter to see the fertilization rate (3 days after AI)

Gr.-C3 (5 sows): to see the pregnancy rate, farrowing rate and litter size

Group-D (15 sows): inseminated by using a **new catheter (the Absolute AMG Series)** with a dose of **3.0 x 10^9** spermatozoa.

Gr.-D1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)

Gr.-D2 (5 sows): slaughter to see the fertilization rate (3 days after AI)

Gr.-D3 (5 sows): to see the pregnancy rate, farrowing rate and litter size

Material and Methods

Oestrous detection and monitoring of ovulation

Oestrous detection was performed by inspection of the vulva for reddening and swelling (prooestrus) as well as by control of the standing reflex (oestrous) in the presence of a boar. The oestrous detection was carried out twice daily. Ovulation was followed every 8 h by trans rectal ultrasonography as described earlier (Kaeoket et al., 2002; Kaeoket et al., 2005).

Insemination and slaughter

All sows were inseminated twice by the same person at 24 h and 36 h after standing oestrous with a dose of pooled semen (two boars of proven fertility), containing 1.5 x 10^9 or 3 x 10^9 spermatozoa in 100 ml BTS (Beltsville Thawing Solution; Pursel and Johnson, 1976). After dilution, the semen was stored at 1618°C and used within 48 h by using a foam tip and a new catheter. Sows were allocated to **slaughter in different groups**.

Group-A (15 sows): inseminated by using **a foam tip** with a dose of **1.5 x 10^9** spermatozoa.

Gr.-A1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)

Gr.-A2 (5 sows): slaughter to see the fertilization rate (3 days after AI)

Group-B (15 sows): inseminated by using a **foam tip** with a dose of **3.0 x 10^9** spermatozoa.

Gr.-B1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)

Gr.-B2 (5 sows): slaughter to see the fertilization rate (3 days after AI)

Group-C (15 sows): inseminated by using a **new catheter (the Absolute AMG Series)** with a dose of **1.5 x 10^9** spermatozoa.

Gr.-C1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)

Gr.-C2 (5 sows): slaughter to see the fertilization rate (3 days after AI)

Group-D (15 sows): inseminated by using a **new catheter (the Absolute AMG Series)** with a dose of **3.0 x 10^9** spermatozoa.

Gr.-D1 (5 sows): slaughter to see the sperm distribution in the uterus and oviduct (5-6 h after AI)

Gr.-D2 (5 sows): slaughter to see the fertilization rate (3 days after AI)

The genital organs were removed immediately after slaughter. The number of corpora lutea were counted.

Recovery of oocytes and spermatozoa from flushed oviducts

The flushing technique allows a more accurate assessment of the number and distribution of oviductal spermatozoa than in situ observation with a scanning electron microscope (Mburu et al., 1996). For that reason, the UTJ (1 cm of the tip of the uterine horn and 1 cm of the isthmus) were flushed twice with 0.5 ml and isthmus and ampulla separately twice with 10 ml of a phosphate buffer saline (PBS) at 37°C (both sides). All the flushing's were made directly into plastic Eppendorf vials (UTJ) or petri dishes (isthmus and ampulla). Spermatozoa from the flushed UTJ were fixed with formal-saline solution and evaluated under the light microscope by using a haemocytometer (Bürker chamber, magnification x400). The oocytes were recovered under a stereomicroscope and examined under an inverted phase contrast microscope (magnification x200) for the

presence of spermatozoa in the zona pellucida. The oviducts (isthmus and ampulla) of sows were also flushed to recover oocytes.

Recovery of unfertilized and cleaved oocytes from flushed uterine horns

The uterine horns (20 cm from the tip of the horns) from sows were flushed twice with 20 ml of phosphate buffer saline (PBS) at 37°C and the fluid was collected in petri dishes. The oocytes were then isolated and examined under a stereomicroscope and an inverted phase contrast microscope (Olympus, Japan; magnification x200) for their morphology and developmental stage. An oocyte was considered as non-fertilized when no cleavage was observed. A cleaved oocyte was considered normal when a clear perivitelline space was seen and the blastomeres were distributed with no sign of disintegration.

Pregnancy rate, farrowing rate and litter size

The pregnancy detection was performed between days 18-21 after insemination by using real time (B-mode) ultrasound.

Farrowing rate and litter size were also recorded.

Statistical analyses

Data were analyzed by using SAS programme (1989). The PROC FREQ (Fisher's exact test, two tails) was used to compare the distribution of spermatozoa, the number of oocytes with accessory sperm to the zona pellucida and fertilized oocytes between a foam tip and a new IUI catheters.

References

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