

Scientific Protocols Used When Evaluating The New Absolute Σ UI™ Catheter Compared To Traditional Foam Catheters

新的 Absolute Σ UI™ 人工授精管与传统的人工授精管相比评估时用的科学议定

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Aims 目的是

- To compare the sperm distribution in the Utero-tubal junction (UTJ) and the oviduct (comparing AI and IUI)
 - To compare fertilisation 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 10⁹ spermatozoa)
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- 比较精子分布在 Utero-tubal Junction (UTJ) 和 输卵管 (比较 AI 和 IUI)
 - 比较受精率 (比较 AI 和 IUI)
 - 比较怀孕率 (比较 AI 和 IUI)
 - 比较产仔率 (比较 AI 和 IUI)
 - 比较产仔数 (比较 AI 和 IUI)
 - 比较人工授精精子的剂量 (1.5 与 3.0 × 10⁹ 精子)

Experimental design 实验设计

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 1.5×10^9 spermatozoa.

共有 60 母猪，分为 4 组（各组 15 头母猪）。他们是从商业农场购买，并保存在 Mahidol 大学。A 组（15 头母猪）：受精是用传统授精管和 1.5×10^9 精子的剂量。

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 fertilisation rate (3 days after AI)

Gr.-A3 (5 sows): to see the pregnancy rate, farrowing rate and litter size

A1 组（5 母猪）：屠宰看精子分布在子宫和输卵管（授精 5-6 小时后）

A2 组（5 母猪）：屠宰看受精率（授精三天后）

A3 组（5 母猪）：看怀孕率，产仔率和产仔数

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

B 组（15 母猪）：受精用传统授精管 3.0×10^9 精子的剂量。

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 fertilisation rate (3 days after AI)

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

B1 组（5 母猪）：屠宰看精子分布在子宫和输卵管（授精 5-6 小时后）

B2 组（5 母猪）：屠宰看受精率（受精三天后）

B3 组（5 母猪）：看怀孕率，产仔率和产仔数

Group-C (15 sows): inseminated by using a new catheter (the Absolute Σ UI™) with a dose of 1.5×10^9 spermatozoa.

C 组（15 母猪）：受精是用新的 Ab Σ UI™ 人工授精管和 1.5×10^9 精子的剂量。

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 fertilisation rate (3 days after AI)

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

C1組 (5 母猪) : 屠宰看精子分布在子宫和输卵管 (授精 5-6 小时后)

C2 組 (5 母猪) : 屠宰看受精率 (受精三天后)

C3 組 (5 母猪) : 看怀孕率, 产仔率和产仔数

Group-D (15 sows): inseminated by using a **new catheter (the Absolute Σ UI™)** with a dose of **3.0 x 10⁹** spermatozoa.

D组 (15 头母猪) : 受精是用新的Ab Σ UI™人工授精管和 3.0×10⁹ 精子的剂量。

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 fertilisation rate (3 days after AI)

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

D1組 (5 母猪) : 屠宰看精子分布在子宫和输卵管 (授精 5-6 小时后)

D2 組 (5 母猪) : 屠宰看受精率 (受精三天后)

D3 組 (5 母猪) : 看怀孕率, 产仔率和产仔数

Material and Methods 材料与amp;方法

Oestrous detection and monitoring of ovulation 发情探测和监测排卵

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

发情检测是由检查外阴为变红和肿胀, 以及由控制在公猪前站立反应 (发情) 存在。该发情检测每日进行两次。排卵检查, 每 8 小时, 由经直肠超声检查作为先前所述 (kaeoket 等人, 2002 年; kaeoket 等人, 2005 年) 。

Insemination and slaughter 受精和屠宰

All sows were inseminated twice by the same person at 24 h and 36 h after

standing oestrus with a dose of pooled semen (two boars of proven fertility), containing 1.5×10^9 or 3×10^9 spermatozoa in 100 ml BTS (Beltsville Thawing Solution; Pursel and Johnson, 1976). After dilution, the semen was stored at 16-18°C and used within 48 h by using a foam tip and a new catheter. Sows were allocated to **slaughter in different groups**.

所有的母猪由同一人接受两次受精,在证实发情后 24 小时和 36 小时;剂量汇集精液(由证明生育的 2 公猪),其中载有 1.5×10^9 或 3×10^9 精子在 100 毫升 BTS 稀释剂液体(Beltsville Thawing Solution; Pursel and Johnson, 1976 年)。经过稀释,精液是储存在 16 - 18 ° C 和在 48 小时内使用于传统或新的导管。母猪分配给屠宰在不同的群体。

Group-A (15 sows): inseminated by using a foam tip with a dose of 1.5×10^9 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 fertilisation rate (3 days after AI)

A组 (15头母猪) : 受精是用传统授精管和 1.5×10^9 精子的剂量。

A1组 (5母猪) : 屠宰看精子分布在子宫和输卵管(授精 5-6 小时后)

A2组 (5母猪) : 屠宰看受精率(授精三天后)

Group-B (15 sows): inseminated by using a **foam tip** with a dose of 3.0×10^9 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 fertilisation rate (3 days after AI)

B组 (15 母猪) : 受精用传统授精管 3.0×10^9 精子的剂量。

B1组 (5母猪) : 屠宰看精子分布在子宫和输卵管 (授精 5-6 小时后)

B2组 (5母猪) : 屠宰看受精率 (受精三天后)

Group-C (15 sows): inseminated by using a **new catheter (the Absolute Σ UI™)** with a dose of 1.5×10^9 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 fertilisation rate (3 days after AI)

C组 (15 母猪) : 受精是用新的Ab Σ UI™人工授精管和 1.5×10^9 精子的剂量。

C1组 (5母猪) : 屠宰看精子分布在子宫和输卵管 (授精 5-6 小时后)

C2组 (5母猪) : 屠宰看受精率 (受精三天后)

Group-D (15 sows): inseminated by using a **new catheter (the Absolute Σ UI™)** with a dose of 3.0×10^9 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 fertilisation rate (3 days after AI)

D组 (15 头母猪) : 受精是用新的Ab Σ UI™人工授精管和 3.0×10^9 精子的剂量。

D1组 (5母猪) : 屠宰看精子分布在子宫和输卵管 (授精 5-6 小时后)

D2组 (5母猪) : 屠宰看受精率 (受精三天后)

The genital organs were removed immediately after slaughter. The number of corpora lutea were counted. 屠宰后, 生殖器官立即被拆除。人数计 corpora lutea 的数。

Recovery of oocytes and spermatozoa from flushed oviducts

从输卵管以冲水式回收卵母细胞和精子

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 flushings 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 unfertilised and cleaved oocytes from flushed uterine horns

从子宫角以冲水式回收不育的卵母细胞

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-fertilised 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.

怀孕率，产仔率和产仔数

怀孕检测授精后 18-21 天之间，用实时（B 型）超声。

产仔率和产仔数亦录。

Statistical analyses 统计分析

Data were analysed 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 fertilised oocytes between a foam tip and a new IUI catheters.

References 参考

- Mburu, J.N., Einarsson, S., Lundeheim, N., Rodriguez-Martinez, H., 1996. Distribution, number and membrane integrity of spermatozoa in the pig oviduct in relation to spontaneous ovulation. *Anim. Reprod. Sci.* 45, 109-121.
- Kaeoket, K., Persson E., Dalin A.-M., 2002. The influence of pre- and post-ovulatory insemination on sperm distribution in the oviduct, accessory sperm to the zona pellucida, fertilisation rate and embryo development in sows. *Anim. Reprod. Sci.* 71, 239-248.
- Kaeoket, K., Tantasuparuk, W, Kunavongkrit, A. 2005. The effect of post-ovulatory insemination on the subsequent oestrous cycle length, embryonic loss and vaginal discharge in sows. *Reprod. Dom. Anim.* 40, 492-494.