The introduction of a double-channel system for the intrafollicular treatment of cattle

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ABSTRACT: The aim of this study was to evaluate a new double-channel system for ultrasound-guided transvaginal intrafollicular treatment in cattle. The system is equipped with separate aspiration and treatment channels facilitating the aspiration of a small part of follicular fluid followed by the immediate injection of the same amount of treatment solution. In Experiment 1 an intracystic injection was performed successfully in all cows (6/6). In Experiment 2 saline was administered to dominant follicles with an overall success rate of 87.5% (14/16). This new double-channel system represents a reliable method for intrafollicular treatment preceded by an aspiration of the necessary amount of follicular fluid without an increase in intrafollicular pressure.

Keywords: transvaginal aspiration; intrafollicular injection; intracystic injection; double-channel device; cow

List of abbreviations

hCG = human chorionic gonadotrophine, **IFT** = intrafollicular treatment, **LH** = luteinising hormone, **OPU** = ovum pick up, **TVFA** = ultrasound guided transvaginal follicular aspiration

Ultrasound guided transvaginal follicular aspiration (TVFA) in cattle has been used for oocyte collection in *in vitro* embryo production (ovum pick-up, OPU) (Pieterse et al. 1988), dominant follicle ablation (Bergfelt et al. 1994), follicular fluid collection (Vos et al. 1994; Leroy et al. 2004) or foetal fluid collection (Vos et al. 1990; Garcia and Salaheddine 1997). After modifications to the TVFA, the equipment has also been used for intraovarian treatment (IFT) (Kot et al. 1995). Intrafollicular injection of different substances such as hCG (Kot et al. 1995), phosphate-buffered saline (Bergfelt et al. 1998) or insulin-like growth factors (Ginther et al. 2004; Shahiduzzaman et al. 2010), has been described. Injection into ovarian stroma is also reported (Oropeza et al. 2004).

The adaptation of instruments used for IFT is similar. A long tool (approximately 50 cm) is inserted in place of an aspiration needle in a metal needle guiding tube in a plastic transducer handle. The channel is fitted with a syringe and completely filled with the treatment solution. The volume of the solution injected varies from 20 to 300 µl (Kot et al. 1995; Ginter et al. 2004; Hanstedt et al. 2011). Although all reports have described a minimal dead volume in the treatment system, none have stipulated the exact volume. Further, if the IFT is not preceded by follicular aspiration, even a minimal amount of injected fluid results in an increase in intrafollicular pressure.

The objective of the study was to evaluate the applicability of the new device for IFT using the double-channel system with standard disposable injection needles. The new system enables the aspiration of follicular fluid and subsequent IFT with exactly the same amount of solution when the dead volume is only the volume of the needle.

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MATERIAL AND METHODS

Equipment for intrafollicular treatment

A convex ultrasound transducer (7.5 MHz Aloka UST 9125, Japan) (1) was placed in a holder (2) at an angle to allow for good visibility of the aspiration needle on the monitor of a real-time B-mode ultrasound scanner (SSD-500, Aloka, Japan). A probe holder of the usual shape and appropriate size was made from two plastic parts connected by screws. A metal guide tube (3) was introduced to the upper part of the ultrasound probe holder. This was used for the insertion and free movement of the needle holder. The needle holder (4) was the main inner part of the device, manipulated by the examiner with a handle (8). An inner part of the needle holder contained two channels (a treatment channel and an aspiration channel) running to the crest of the conical end of the holder, both leading in a single injection needle (5). Standard disposable Luer-type needles were used for IFT (0.65 × 40 mm, 22 G). At the rear end of the holder the channels were terminated by Luer connections (7) where an injection set (6) was attached. The injection set consisted of two shortened infusion tubes (11), and two shortened injection needles (9) equipped with thickened ends (10) were inserted into the tubes along with two 1 ml syringes (12). In the first tube, a needle and syringe were used for aspiration and in the second they were used for treatment. All parts of the new device were manufactured by MEDIN, a.s., Nove Mesto, Czech Republic (Figure 1, 2).

Intrafollicular treatment protocol

The treatment syringe was filled with a solution and an injection set was completed. The treatment tube and treatment channel were completely filled with the treatment solution (0.45 ml) by moving the syringe plunger. An injection needle was also attached at the conical end of the needle holder.

Animals received epidural anaesthesia (5 ml of 2% lidocaine; Lidocaine 2%, Fatro, Italy) to prevent straining during aspiration. After emptying the rectum, the vulva and perineal area were thoroughly cleaned and disinfected. The probe holder was inserted deep into the fornix vaginae until the vaginal wall tightened. An examiner manipulated the ovary through the rectal wall and located the target follicular structure on the scanner screen. The needle holder with completed injection set was inserted into a guide tube, and the examiner inserted the needle into the centre of the target structure by manipulating the end of the syringe holder. An assistant aspirated a sufficient amount of follicular fluid (0.2 ml) into the aspiration syringe and immediately injected the same amount of treatment solution by advancing the injection syringe plunger. The moment of injection was visible on the scanner screen as a turbulence of fluid inside the follicular cavity.

Experimental design

Experiment 1: Evaluation of applicability of the double-channel device for intracystic injection. Cows bearing ovarian cysts of a diameter of at least 25 mm (n = 6) were treated by intracystic injection with hCG (500–1000 IU *pro toto*; Pregnyl inj. 3×1500, N.V. Organon, Netherlands). After a course of injections changes in the cystic wall seven days after IFT were evaluated.

Experiment 2: Evaluation of applicability of the double-channel device for intrafollicular injection. Experimental cows (n = 12) in the luteal phase were

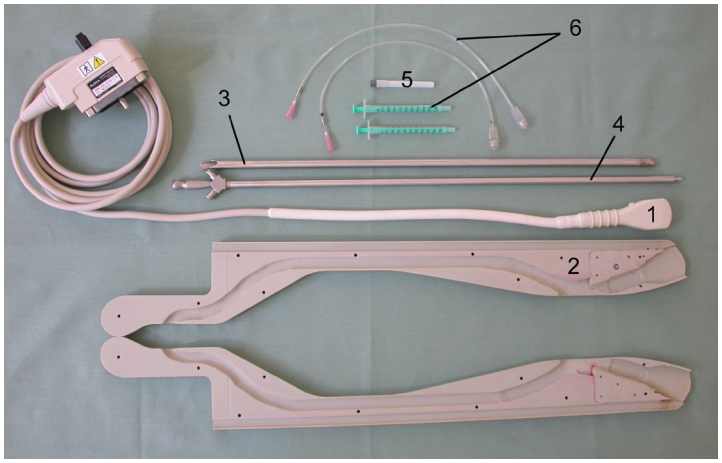

Figure 1. The entire set of equipment used for intrafollicular treatment in cattle. An ultrasound transducer (1), a holder (2), a metal guide tube (3), a needle holder (4), a disposable injection needle (5), an injection set (6)

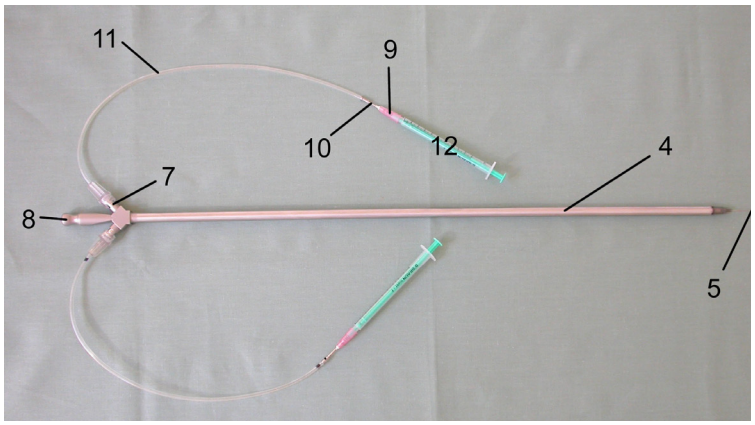

Figure 2. A detailed depiction of the syringe holder with the injection set attached. A needle holder (4), disposable injection needle (5), two Luer connections (7), handle (8), shortened injection needless (9), thickened ends of shortened needless (10), shortened infusion tubes (11), syringes (12)

synchronised using cloprostenol (500 µg *i.m. pro toto*; Oestrophan®, Bioveta, a.s., Czech Republic) (Day –3). Gonadorelinum (50 µg *i.m. pro toto*; Depherelin Gonavet, Veyx, Germany) was administered 48 h later (Day –1). Intrafollicular treatment with 0.2 ml of saline was performed on 16 follicles (two follicles were injected in four cows) on Day 5 using 22G needles (0.65 × 40 mm), cloprostenol was administered on Day 7. An ultrasound examination was performed daily in order to observe ovulation (Day 1) after the first cloprostenol administration, subsequent corpus luteum development and the first follicular wave until IFT, over the course of IFT, and further up until Day 16. A scanner Aloka SSD-500 equipped with linear 7.5 MHz transducer UST 5561 – 7.5 was used; documentation was made using a Sony camera DCR-TRV235E. The course of IFT, the condition of follicles after IFT, their subsequent development and number of ovulations after the second cloprostenol treatment were all recorded (Table 1).

RESULTS

Experiment 1

Intracystic injection was performed successfully in all six cows. The needle was visible as a hyperechogenic line across the cyst cavity; a clear flow of treatment solution passing into echogenic turbulence was observed after the syringe piston was advanced. Changes in cyst structure seven days after IFT were slight, only an irregular thickening of the cystic wall was observed.

Table 1. Intrafollicular treatment in cows: treatment schedule

Day	Treatment
–3	cloprostenol
–1	GnRH
5	IFT
7	cloprostenol

Experiment 2

Saline (0.2 ml) was administered to 16 follicles in 12 cows. The tip of the penetrating needle and subsequent swirling of follicular fluid in the follicular cavity was observed in all cases. Two intrafollicular treatments were evaluated as unsuccessful because the treated follicles were smaller 24 h later and further growth did not ensue. Overall, the success rate of IFT was 87.5% (14/16).

Other follicles (n = 14) showed slight variations in diameter 24 h after IFT (12.6 mm vs. 11.7 mm); however, they continued to develop. These reached preovulatory size after cloprostenol treatment (14–16.7 mm) and some cows showed oestrus signs; however, only four cows ovulated. The remaining follicles (n = 10) persisted until the last examination on Day 16.

DISCUSSION

Intraovarian treatment was first described almost 60 years ago (Paredis and Vandeplassche 1953). At that time, the technical possibilities allowed only intracystic treatment by hCG (Roberts 1957; Holy and Kudelka 1958; Buhner and Liebetrau 1963; Pepper 1973). This method has not been used in practice; however, this insight could be built upon later.

The development of ultrasound guided transvaginal follicular aspiration essentially led to the revi-

talisation of intraovarian treatment. Intraovarian injection of different substances has been performed in cattle. A two needle system (outer needle for perforation of vaginal wall and ovarian stroma and an inner needle for puncturing the follicular wall) (Kot et al. 1995; Ginther et al. 2004) or a one needle system (Bergfelt et al. 1998; Oropeza et al. 2004; Hanstedt et al. 2011; Lopez-Gatius and Hunter 2011), have been described. Descriptions of the instruments are not detailed; however, all instruments described use a one-way system with some-according to the authors, "minimal"-dead volume. IFT without previous aspiration of the equivalent amount of follicular fluid (Kot et al. 1995; Hanstedt et al. 2011), and undertaken without problems caused by leakage of follicular fluid due to an increase in intrafollicular pressure has been reported. In our opinion, even the injection of a minimal volume of treatment solution results in an increase in intrafollicular pressure, mainly depending on the size of the follicle. This results in a higher risk of leakage of follicular fluid or even follicular rupture.

It is interesting that the same problems were solved by scientists during intracystic treatment many years ago (Holy and Kudelka 1958). According to these researchers it was necessary to firstly remove part of the cyst's content to prevent an increase in intracystic pressure during the refilling of the cyst with the treatment solution. Otherwise intracystic treatment could lead to the cyst destruction or at least to leakage of cystic fluid (Holy and Kudelka 1958). The same physical laws are valid in cases of intrafollicular injection as well; however, the size of the treated structures and the volume of solution used were significantly lower.

The influence of mechanical features of aspiration equipment on increases in intrafollicular pressure during oocyte collection has been documented. An influence of the bevel shape of aspiration needles has been described, with regard to the leakage of follicular fluid and traumatisation of the follicular wall. Those features have been described during laparoscopic follicular aspirations (Fayrer-Hosken and Caudle 1991); however, they are valid irrespective of whether laparoscopic or transvaginal access to ovaries is used. Increasing intrafollicular pressure after intrafollicular treatment without previous aspiration has not been measured; nevertheless, it occurs even after a minor follicular puncture preceding oocyte aspiration (Horne et al. 1996).

Therefore, we devised a new double-channel system equipped with separate aspiration and treatment channels facilitating the aspiration of a small part of the follicular fluid (if necessary all follicular fluid when biochemical analysis is required) followed by the immediate injection of the same amount of treatment solution from the treatment syringe. Thus, intrafollicular pressure will not be increased by IFT. Both aspiration and treatment channels lead to the conical end of the needle holder in a single disposable needle. Both channels were constantly closed by syringes and switching from the aspiration to the treatment phase was very fast.

In Experiment 1 the intracystic injection of hCG was performed successfully in all six cows. Replacement of a definite volume of fluids without damage to the treated structure was considered as a successful injection. The first use of the instrument proved that the movement of fluid managed by the syringes is flowing according to our assumptions. The response of the cystic wall to the hCG was slight; however this was not the main focus of the study. This treatment will be applied to higher numbers of cystic animals with different doses of hCG in the future.

In Experiment 2 0.2 ml of saline were administered to dominant follicles in D5 and this was performed using the standard 22G needles (diameter 0.65 mm). A high success rate of the treatment (which was demonstrated by subsequent growth) was observed (14/16, 87.5%); only two treated follicles showed a considerable decrease in diameter 24 h after the treatment. Those two attempts were evaluated as unsuccessful.

According to the available data (Bergfelt et al. 1998), a slight decrease in diameter (1–3 mm) has been observed in 67% of treated follicles. Only in 33% of treated follicles was an increase in diameter observed 12 h after IFT; however, this was noticed in 83% of animals 24 h after IFT (Bergfelt et al. 1998). Regardless of the slight leakage of follicular fluid, the treated follicles were able to develop and finally ovulate. Ovulation occurred as early as 12 h after IFT in one animal and the authors speculated that follicle puncture may have hastened or induced ovulation (Bergfelt et al. 1998). In another study (Kot et al. 1995), a reduction in follicle diameter exceeding 1–2 mm was the reason for the removal of experimental animals from the study.

In our study successfully treated follicles (n = 14) showed a slight variation in diameter 24 h after IFT; however, in subsequent examinations they

continued to grow, after cloprostenol treatment until reaching their preovulatory size. We can only guess as to why only four of all successfully treated follicles ovulated. Luteolysis induced by cloprostenol administration on D7 was clearly demonstrated by ultrasound examination; the development of treated follicles was very progressive and cows showed oestrogenisation at the time of expected oestrus. We speculate that the observed effects could be the result of follicle traumatisation leading to a disturbance in final follicular wall maturation, because in comparison with other studies (Kot et al. 1995; Ginther et al. 2004; Hanstedt et al. 2011), we used needles with a larger diameter (22G, 0.65mm). Another reason could be a disturbance in feed-back mechanisms caused by the aspiration of a part of the follicular fluid with subsequent LH surge failure; however, without an endocrinological examination we cannot be certain that this was the case. Although culled cows have been used for experiments (they could have shown ovulation failure even without IFT), we can eliminate this as a reason. As we knew the gynaecologic anamnesis, cows with a history of cystic ovarian disease were excluded. Further, the selected animals were proven to have the ability to ovulate twice. Firstly, the cows ovulated during the pre-selection for Experiment 2, when only cows bearing a corpus luteum were included and secondly, after the first cloprostenol treatment (Day –3) was performed for synchronization. The second ovulations and subsequent CL development were monitored frequently by ultrasound examinations. Therefore, we do not have a clear explanation for the observed ovulation failures. It will be necessary to perform the experiments on a higher number of animals, with endocrinological examinations and with needles of different sizes.

We conclude that the new double-channel system for intrafollicular treatment in cattle is fully functional. It represents a reliable method for intrafollicular treatment preceded by an aspiration of the necessary amount of the follicular fluid, without an increase in intrafollicular pressure.

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